

APPLICATION

FOR

UNITED STATES LETTERS PATENT

NOVEL EXPRESSION VECTORS CONTAINING ACCESSORY  
MOLECULE LIGAND GENES AND THEIR USE FOR  
IMMUNOMODULATION AND TREATMENT OF  
MALIGNANCIES AND AUTOIMMUNE DISEASE

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DESCRIPTION

NOVEL EXPRESSION VECTORS CONTAINING ACCESSORY MOLECULE  
LIGAND GENES AND THEIR USE FOR IMMUNOMODULATION AND  
TREATMENT OF MALIGNANCIES AND AUTOIMMUNE DISEASE

RELATED APPLICATION

This application claims priority to Kipps et al.,  
NOVEL EXPRESSION VECTORS CONTAINING ACCESSORY MOLECULE  
5 LIGAND GENES AND THEIR USE FOR IMMUNOMODULATION AND  
TREATMENT OF MALIGNANCIES, United States Provisional  
Application No. 60/032145, filed December 9, 1996, which  
is incorporated herein by reference including drawings.

10 TECHNICAL FIELD OF THE INVENTION

The present invention relates to novel expression  
vectors containing genes which encode an accessory  
molecule ligand and the use of those vectors for  
15 immunomodulation, improved vaccination protocols and the  
treatment of malignancies and autoimmune diseases. More  
particularly, this invention provides expression vectors  
and methods for treating various neoplastic or malignant  
cells, and expression vectors and methods for treating  
20 autoimmune Disease. This invention also contemplates  
the production and expression of accessory molecule  
ligands with greater stability and enhanced function.

25 BACKGROUND OF THE INVENTION

Leukemias, lymphomas, carcinomas and other  
malignancies are well known and described in, e.g.,  
Harrison's Principles of Internal Medicine, Wilson et  
al., eds., McGraw-Hill, New York, pp. 1599-1612. These

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malnancies appear to have somehow escaped the immune system surveillance mechanisms that eliminate rapidly and continuously proliferating cells. The exact mechanism by which these malignancies escape the immune system surveillance is not known.

Some of these malignant immune system cells are malignant antigen presenting cells which do not function properly within the immune cascade. For example, neoplastic B cells cannot induce even weak allogeneic or autologous mixed lymphocyte reactions in vitro. Further evidence that malignancies survive due to the failure of the immune surveillance mechanism includes the increased frequency of such malignancies in immunocompromised individuals, such as allograft recipients and those receiving long-term immunosuppressant therapy. Further, the frequency of these malignancies is increased in patients having Acquired Immune Deficiency Syndrome (AIDS) and patients with primary immune deficiency syndromes, such as X-linked lymphoproliferative syndrome or Wiscott-Aldrich Syndrome (Thomas et al., Adv. Cancer Res. 57:329, 1991).

The immune system normally functions to eliminate malignant cells by recognizing the malignant cells as foreign cells and clearing those cells from the body. An immune reaction depends on both the immune system's antibody response and on the cellular immune response within a patient. More specifically, the cellular immune response which acts to recognize the malignant cells as foreign requires a number of different cells of the immune system and the interaction between those cells. An immune reaction begins with a T lymphocyte (T cell) which has on its cell surface the T cell receptor. The T cell also has the ability to express on its surface various accessory molecules which interact with accessory molecules on the B lymphocyte (B cell). When

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the T cell receptor of the T cell specifically binds to a foreign antigen, such as a malignant cell, it becomes activated and expresses the accessory molecule ligand, CD40 ligand on its cell surface. The accessory cell molecule ligand is only present on the activated T cells for a short period of time and is rapidly removed from the cell surface. After the accessory cell molecule ligand is removed from the surface of the activated T cell, its ability to bind to B cells via the accessory molecule ligand is destroyed.

When present on the surface of an activated T cell, the accessory cell ligand can specifically bind to the accessory cell molecule present on the B cell. This specific T-B cell interaction causes the B and T cell to express <sup>Costimulatory</sup> ~~costimulatory~~ surface accessory molecule and cytokines which result in an immune activation which lead to cytolytic T cells which specifically kill and remove the malignant cell from the body.

The interaction with an activated T cell is not solely limited to B cells but rather can be carried out by any cell which is able to present antigen to the T cell (an antigen presenting cell). These cells include B lymphocyte, macrophages, dendritic cells, monocytes, Langerhans cells, interdigitating cells, follicular dendritic cells or Kupffer cells. These cells all are known to have various accessory molecules on the cell surface which allow them to interact with other cells of the immune system. For example, these antigen presenting cells all have the accessory molecule CD40 on their cell surface. The presence of these accessory molecules allows these antigen presenting cells to specifically bind to complimentary accessory molecule ligand and thus directly interact with other immune cells.

A large number of accessory molecule ligands are members of the tumor necrosis factor superfamily. (Fanslow et al., Sem. Immun., 6:267-268 (1994). The genes for a number of these accessory molecule ligands have been cloned and identified. These accessory molecule ligand genes encode accessory molecules which all have the configuration of Type II membrane proteins and exhibit varying degrees of homology with other accessory molecule ligand genes. For example, the accessory molecule ligand genes encoding both murine CD40 ligand and human CD40 ligand have been isolated. See, Armitage et al., Nature, 357:80-82 (1992) and Hollenbaugh et al., EMBO J., 11:4313-4321 (1992).

CD40 and its ligand, CD40 ligand are critical components of a normal immune response. CD40 mediated signals induce immune lymphocytes to proliferate and differentiate and become potent antigen presenting cells. Malignant or neoplastic B cells are poor antigen presenter cells and are unable to stimulate a vigorous allogeneic mixed lymphocyte reaction. Successful cross linking of CD40 molecules on immune cells results in a strong allogeneic mixed lymphocyte reaction suggesting a strong immune reaction. Various soluble CD40 ligands or antibodies specific for CD40 have been used to potentially cross link CD40. These soluble CD40 ligands and CD40-specific antibodies are not optimal for cross linking the CD40 molecules on antigen presenting cells and do not work as effectively as CD40 ligand expressed on a cell membrane to produce strong stimulation of antigen presenting cells. These methods are also difficult to implement because large amounts of CD40 ligand constructs or antibodies must be isolated which is difficult and time-consuming work. Other strategies to utilize CD40 ligand in solution or as a membrane bound molecule including transformation of fibroblasts

with CD40 ligand to produce cultured cells which are then used to present antigen are not amenable to in vivo human clinical protocols.

CD95 (Fas) interaction with its ligand (Fas-ligand, or FasL) functions to limit the duration of the immune response and/or life-span of activated lymphocytes. Apoptosis induced by Fas-FasL binding serves to clear activated self-reactive lymphocytes. Problems caused by altering this pathway have been demonstrated in animals with defects in Fas<->Fas-ligand interactions. Mice having mutations, which inactivate CD95 or FasL, develop numerous disorders including autoimmune pathology resembling that seen in patients with rheumatoid arthritis (RA) or systemic lupus. Zhang, et al., in J. Clin. Invest. 100:1951-1957 (1997) show that injection of FasL-expressing virus, into the joints of mice with collagen-induced-arthritis, results in apoptosis of synovial cells and relief of arthritis symptoms. Expression of Fas ligand allows clearance of activated cells which play a role in the pathogenesis of autoimmune disease. Therefore, a gene therapy strategy for introducing FasL into the joints of rheumatoid arthritis patients could function to improve disease pathology by leading to destruction of the infiltrating mononuclear cells.

Administration of soluble accessory molecules and accessory molecule ligands has been shown to trigger or to be associated with adverse physiological effects. For example, treatment of mice, having wild-type CD40-receptor expression, with soluble CD40L-CD8 fusion protein resulted in a pulmonary inflammatory response. This was not observed in mice in which the gene for the CD40 receptor had been knocked out. These experiments, described in Wiley, J.A. et al., Journal of Immunology 158:2932-2938 (1997), support in vitro data which

suggest that CD40 ligation can result in inflammatory responses.

Direct administration of purified recombinant soluble Tumor Necrosis Factor (either  $\alpha$  or  $\beta$ ) results in shock and tissue injury, as described in Tracey, K. J., and A. Cerami, Annu. Rev. Med. 45:491-503 (1994). Within minutes after acute intravenous or intra-arterial administration of TNF, a syndrome of shock, tissue injury, capillary leakage syndrome, hypoxia, pulmonary edema, and multiple organ failure associated with a high mortality ensues. Chronic low dose of TNF causes anorexia, weight loss, dehydration and depletion of whole-body protein and lipid.

Soluble Fas ligand and receptor have also been shown to be associated with tissue damage and other adverse effects. CD95, the Fas receptor, is a mediator of apoptosis. Fas ligand induces apoptosis by binding to Fas receptor. As shown in Galle, P.R., et al., J. Exp. Med. 182:1223-1230 (1995) administering an agonistic anti-Fas antibody resulted in liver damage to mice. Mice injected intraperitoneally with the agonistic antibody died within several hours, and analyses revealed that severe liver damage by apoptosis was the most likely cause of death.

The role of soluble Fas ligand (FasL), in the pathogenesis of systemic tissue injury in aggressive lymphoma is described in Sato, K. et al., British Journal of Haematology, 94:379-382 (1996). The findings presented in this report indicate that soluble FasL is directly associated with the pathogenesis of liver injury and pancytopenia.

CD27, the receptor for the accessory molecule ligand, CD70, was shown, in a report written by van Oers, et al., in Blood 82:3430-3436 (1993), to be associated with B cell malignancies.

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The above findings all contraindicate the administration of soluble accessory molecule ligands, highlighting the need for therapies that increase the levels of these molecules without resulting in an elevation of their soluble forms.

Despite the wealth of information regarding accessory molecule ligand genes and their expression on the surface of various immune cells, the exact mechanism by which the accessory molecule ligand genes are regulated on antigen presenting cells is not yet known. Without specific knowledge of the regulation of expression of accessory molecule ligand genes on these antigen presenting cells, altering the immune response by varying expression of an accessory molecule ligand gene has to date not been possible. Without any specific knowledge as to how to regulate the expression of an accessory molecule ligand gene on an antigen presenting cell, it is not possible to alter the immune response towards malignant cells. Thus, there was a need for a method of increasing the expression of an accessory molecule ligand gene on normal and malignant cells including antigen presenting cells.

Further, without the ability to regulate the expression of accessory molecule ligands, it is not possible to alter the immune clearance of these cells.

#### SUMMARY OF THE INVENTION

The present invention fills these needs by providing novel expression vectors containing accessory molecule ligand genes and methods for introducing those genes into normal and malignant antigen presenting cells thereby allowing the alteration of an immune response, the treatment of autoimmune diseases and the treatment of various neoplasias. This invention provides vectors,



including gene therapy vectors which contain accessory molecule ligand genes. These vectors also contain the additional genetic elements, such as promoters, enhancers, polyadenylation signals (3' ends), which  
5 allow that vector to be successfully placed within the cell and to direct the expression of the accessory molecule ligand gene in a cell. Such gene therapy vectors are capable of transforming animal cells directly and thereby introducing the accessory molecule  
10 ligand gene into the cells of that animal in a form which can be utilized to produce accessory molecule ligands within that cell.

In other aspects of the present invention, the function of an accessory molecule ligand is modified by  
15 altering the half life of the molecule on the cell surface or by changing the level of expression of that molecule on the cell surface. In preferred embodiments, the present invention provides accessory molecule ligands which are modified to improve the stability of  
20 such accessory molecule ligands on the cell surface. Such increased stability may be accomplished using any of the disclosed methods of molecules described in this application, including chimeric molecules and molecules into which mutations have been introduced at least one  
25 location. The present invention also contemplates increasing the expression of such a molecule.

The present invention also provides gene therapy vectors containing the accessory molecule ligand genes which are chimeric in that portions of the gene are  
30 derived from two separate accessory molecule ligands which may or may not be from different species. The accessory molecule ligand genes of the present invention include genes which encode molecules of the tumor necrosis factor (TNF) family. The molecules which make  
35 up the TNF family include  $TNF_{\alpha}$ ,  $TNF_{\beta}$ , CD40 ligand, Fas

ligand, CD70, CD30 ligand, 41BB ligand (4-1BBL), nerve growth factor and TNF-related apoptosis inducing ligand (TRAIL). In some embodiments of the present invention, the chimeric accessory molecule ligand genes of the present invention contain at least a portion of a murine accessory molecule ligand gene together with portions of accessory molecule ligand genes derived from either mouse, humans or other species. Some preferred embodiments of the present invention utilize murine CD40 ligand genes and chimeric CD40 ligand genes containing at least a segment of the murine CD40 ligand gene together with at least a segment of the human CD40 ligand gene. The present invention contemplates chimeric accessory molecule ligand genes wherein segments from the accessory molecule ligand gene of one species have been interchanged with segments from a second accessory molecule ligand gene which may optionally be from a different species. For example, in one preferred embodiment, the murine CD40 ligand gene transmembrane and cytoplasmic domains have been attached to the extracellular domains of human CD40 ligand gene.

The present invention contemplates gene therapy vectors which are capable of directly infecting the human, mammal, insect, or other cell. The use of such gene therapy vectors greatly simplifies inserting an accessory molecule ligand gene into those cells. The contemplated gene therapy vectors may be used in vivo or in vitro to infect the desired cell and are particularly useful for infecting malignant cells to effect sustained high-level expression of a physiologic ligand.

The present invention also contemplates animal, mammal, and human cells containing a gene therapy vector which includes an accessory molecule ligand gene and sufficient genetic information to express that accessory molecule ligand within that cell. In preferred

embodiments, the present invention also contemplates human neoplastic antigen presenting cells which contain the gene therapy vectors of the present invention or contain an accessory molecule ligand gene together with a promoter and 3' end region.

The present invention also contemplates human cells and human neoplastic cells containing a gene therapy vector which includes a chimeric accessory molecule ligand gene. The present invention also contemplates bacterial cells or animal cells containing accessory molecule ligand genes, chimeric accessory molecule ligand genes, murine accessory molecule ligand genes, human accessory molecule ligand genes, the gene therapy vectors of the present invention, the vectors of the present invention, and a chimeric accessory molecule ligand gene together with a heterologous promoter, enhancer or polyadenylation sequence.

The present invention also contemplates methods of altering immune response within a human patient or the immunoreactivity of human cells in vivo by introducing a gene which encodes an accessory molecule ligand gene into the human cells so that that accessory molecule ligand is expressed on the surface of those human cells. This method includes the introduction of the accessory molecule ligand gene as part of a gene therapy vector or in association with a heterologous or native promoter, enhancer or polyadenylation signal. Some preferred embodiments of the present invention utilize introduction of Fas ligand genes and chimeric Fas ligand genes, constructed as contemplated above for CD40, into human cells to alter their immunoreactivity. The present invention also includes methods in which such accessory molecule ligand genes are inserted into cells which have the accessory molecule to which the accessory

molecule ligand binds on the surface of the cell into which the accessory molecule ligand gene.

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The present methods of altering immunoreactivity are applicable to all types of human, animal, and murine cells including human neoplastic cells such as human lymphomas, leukemias and other malignancies. In preferred embodiments, this method is used to introduce the gene encoding the accessory molecule ligand into potential antigen presenting cells of a human patient or cell which can stimulate bystanding antigen presenting cells. Such antigen presenting cells include monocytes, macrophages, B cells, Langerhans cells, interdigitating cells, follicular dendritic cells, Kupffer cells, and the like. The various antigen presenting cells may be present as part of a known malignancy in a human patient such as leukemias, lymphomas, acute monocytic leukemia (AML), chronic lymphocytic leukemia (CLL), acute myelomonocytic leukemia (AMML), chronic myelogenous or chronic myelomonocytic leukemia (CMML) and thus would include all tumors of any cell capable of presenting antigen to the human or animal immune system or are capable of stimulating bystanding antigen presenting cells. The present invention also contemplates modulating the immune system by introducing genes encoding an accessory molecule ligand gene of the present invention into any number of different cells found in a patient, including muscle cells, skin cells, stromal cells, connective tissue cells, fibroblasts and the like.

The present invention also contemplates methods of treating neoplasias in either a human patient or an animal patient. In one preferred embodiment, the method comprises isolating the neoplastic cells from the human or animal patient and inserting into those isolated cells the gene which encodes the chimeric accessory

5 molecule ligand or the accessory molecule ligand so that that molecule is expressed on the cell surface of those neoplastic cells or other somatic cells. The neoplastic cells are then infused back into the human or animal patient and may then participate in an enhanced immune response.

10 The present invention also contemplates the co-infection or co-introduction of the accessory molecule ligand gene together with a gene which encodes a tumor or carcinoma specific antigen. This combination of molecules are then expressed on the surface of the neoplastic cells and when those cells are introduced into the patient lead to the rapid immune response resulting in the destruction of those cells.

15 The present methods also include directly introducing the gene therapy vector or other vector carrying the accessory molecule ligand gene directly into the tumor or tumor bed of a patient. Upon entering the tumor bed of the patient, the gene therapy vector or other vector enter the cells present in the tumor or tumor bed and then express the accessory molecule ligand gene on the surface of those cells. These cells then are able to participate fully in the human immune or animal immune response.

20 The present invention also contemplates methods of augmenting an immune response to a vaccine. The present method of vaccinating an animal against a predetermined organism or antigen by administering to that animal a vaccine which has a genetic vector containing an accessory molecule ligand gene. Other embodiments of the present invention include vaccinating an animal by administering two separate genetic vectors, one containing the antigens from the organism to which immunity is desired by isolating the cells of the target animal and contacting with those cells a vector encoding

at least one antigen from a predetermined organism so that the antigen is expressed by the cells and also contacting those cells with a different vector which expresses the accessory molecule ligand gene on the surface of the animal's antigen presenting cells. Together these two separate vectors produce a vaccination which is much stronger and of longer duration than is vaccination with antigen alone.

The present methods of vaccination are applicable to vaccinations designed to produce immunity against a virus, a cell, a bacteria, any protein or a fungus. The present methods are also applicable to immunization against various carcinomas and neoplasias. In these embodiments, the tumor antigen against which immunity is desired is introduced into the animal together with the genetic vector containing the accessory molecule ligand gene.

The present invention also contemplates methods of treating arthritis utilizing a gene therapy vector encoding an accessory molecule ligand. Of particular interest for use with arthritis is the Fas ligand molecule in which the expression of Fas ligand activity has been increased in the joint and/or the stability of the Fas ligand activity on cells within the joint enhanced. In other embodiments, the present invention contemplated methods of treating arthritis utilizing chimeric accessory molecule ligands and chimeric accessory molecule ligand genes. The present invention also contemplates both ex vivo therapy and in vivo therapy of arthritis utilizing the expression vectors of the present invention together with the Fas ligand and modified versions of that molecule including chimeric molecules.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Figure 1 is a diagram showing a number of accessory molecule ligand genes and Domains I-IV of those genes as deduced from sequence data.

Figure 2. Figure 2 is a diagram showing example chimeric accessory molecule ligand genes. The domains derived from the murine accessory module are shown shaded.

Figure 3. Figure 3 shows the amount of either mouse or human CD40 ligand found on the surface of Hela or CLL cells infected with gene therapy vectors containing the genes encoding these molecules. Figure 3A shows uninfected Hela cells (shaded) and Hela cells infected with a gene therapy vector encoding murine CD40 ligand. Figure 3B shows uninfected Hela cells (shaded) and Hela cells infected with a gene therapy vector encoding human CD40 ligand. Figure 3C shows uninfected CLL cells (shaded) and CLL cells infected with a gene therapy vector encoding murine CD40 ligand. Figure 3D shows uninfected CLL cells (shaded) and CLL cells infected with a gene therapy vector encoding human CD40 ligand.

Figure 4. Figure 4 shows histograms of the increased expression of CD54 (Figure 4B) and CD80 (Figure 4D) on CLL cells into which a gene therapy vector containing the accessory molecule ligand gene (murine CD40 ligand gene) has been introduced. The shaded graph indicates control stain in FACS analysis and the open graph indicates staining with monoclonal antibodies immunospecific for either CD54 (Figures 4A and 4B) or CD80 (Figures 4C and 4D).

Figure 5. Figure 5 shows the cell proliferation as measured by <sup>3</sup>H-TdR incorporation of allogeneic T cells in response to various stimulation regimes. The CLL cells

5        Figure 6. Figure 6 shows the production of gamma interferon (IFN $\gamma$ ) by allogeneic T cells stimulated with CLL cells containing an accessory molecule ligand gene.

Figure 8. Figure 8 shows the production levels and stabilities of CD40 ligand and CD40 ligand transcript in CLL (upper graph) and normal blood mononuclear cells (lower graph).

Figure 10. Figure 10 shows changes in surface antigen phenotype of CLL B cells infected with a gene therapy vector containing an accessory molecule ligand, CD40 ligand. Shaded histograms represent staining of



uninfected CLL cells (thin lines) stained with nonspecific control antibody, open histograms drawn with thin lines represent uninfected CLL cells stained with FITC-conjugated specific mAb, and open histograms drawn with thick lines (labeled CD154-CLL) represent CLL cells infected with the accessory molecule ligand gene therapy vector and stained with FITC-conjugated specific mAb.

Figure 11. Figure 11 shows levels of CD27 produced in CLL cells infected with a gene therapy vector containing an accessory molecule ligand. Figure 11A shows that CD40L-infected CLL (CD154-CLL) cells express reduced levels of surface CD27. Open histograms represent staining of non-infected CLL cells (thin lines) or infected CLL (thick lines) with FITC-conjugated  $\alpha$ CD27 mAb, respectively. Figure 11B shows production of soluble form of CD27 by CLL B cells.

Figure 12. Figure 12 shows allogeneic T cell responses induced by CLL cells infected with a gene therapy vector containing an accessory molecule ligand (CD40 ligand, also called CD154). Figure 12A indicates the concentration of IFN $\gamma$  in the supernatants after stimulation of allogeneic T cells with CLL cells containing the accessory molecule ligand. Figure 12B shows cell proliferation, as assessed by incorporation of  $^3$ H-thymidine. Figures 12C and 12D show secondary allogeneic T cell responses induced by CLL containing the accessory molecule ligand.

Figure 13. Figure 13 depicts autologous T cell responses induced by CLL B cells containing the accessory molecule ligand, CD40 ligand or CD154, and controls. Figure 13A shows incorporation of  $^3$ H-thymidine by autologous T cells co-cultured with the CLL cells. Figure 13B shows the levels of human IFN $\gamma$  produced by autologous T cells co-cultured with the CLL cells. In Figure 13C, the CTL activities of autologous T cells

induced by CLL B cells containing the accessory molecule ligand are graphed.

Figure 14. Figure 14 shows specificity of CTL for autologous CLL B cells. IFN $\gamma$  concentration was assessed in the supernatants after 48 h of culture (Figure 14A), and cytolytic activity was assessed at 3 h of culture (Figure 14B). In Figure 14C, mAb were added to the autologous leukemia target cells prior to the CTL assay.

Figure 15. Figure 15 shows that intercellular stimulation plays a role in production of the phenotypic changes observed in CLL cells expressing the accessory molecule ligand. In Figure 15A, the effect of culture density on the induced expression of CD54 and CD80 following infection with a gene therapy vector containing the accessory molecule ligand (CD40 ligand, CD154) is shown. Shaded histograms represent staining of leukemia B cells with a FITC-conjugated isotype control mAb. Open histograms represent CD154-CLL B cells, cultured at high or low density (indicated by arrows), and stained with a FITC-conjugated mAb specific for CD54 or CD80. Figure 15B shows inhibition of CD154-CLL cell activation by anti-CD154 mAb. Figures 15C and 15D depict expression of immune accessory molecules on bystander non-infected CLL B cells induced by CLL cells expressing the accessory molecule ligand. Shaded histograms represent staining with PE-conjugated isotype control mAb.

Figure 16. Figure 16 shows that the vector encoding an accessory molecule ligand enhances immunization against  $\beta$ -gal in mice. Figure 16A shows that mice that received intramuscular injections of the pCD40L vector produced significantly more antibodies to  $\beta$ -gal than did mice injected with either the non-modified pcDNA3 vector or pCD40L. Figure 16B, ELISA analyses of serial dilutions of sera collected at d28, shows that mice co-

injected with placZ and pCD40L had an eight-fold higher mean titer of anti- $\beta$ -gal antibodies at d28 than mice treated with placZ + pcDNA3.

Figure 17. Figure 17 shows analysis of the IgG<sub>1</sub> and IgG<sub>2a</sub> immune responses to intramuscular plasmid DNA immunizations with and without a vector, pCD40L, encoding an accessory molecule ligand. IgG<sub>2a</sub> anti- $\beta$ -gal antibodies predominated over IgG<sub>1</sub> subclass antibodies in the sera of mice injected with either placZ and pcDNA3 or placZ and pCD40L. In contrast, BALB/c mice injected with  $\beta$ -gal protein developed predominantly IgG<sub>1</sub> anti- $\beta$ -gal antibodies, and no detectable IgG<sub>2a</sub> anti- $\beta$ -gal antibodies.

Figure 18. Figure 18 shows the comparison between injection of mice with a vector, pCD40L, encoding an accessory molecule ligand, at the same and different sites as placZ. Adjuvant effect of pCD40L requires co-injection with placZ at the same site.

Figure 19. Figure 19 shows that co-injection into dermis of a vector encoding an accessory molecule ligand, pCD40L, with placZ enhances the IgG anti- $\beta$ -gal response in BALB/c mice.

Figure 20. Figure 20 shows that a vector encoding an accessory molecule ligand, pCD40L, enhances the ability of placZ to induce CTL specific for syngeneic  $\beta$ -gal-expressing target cells. Splenocyte effector cells, taken from mice which had received injections of placZ and pCD40L, specifically lysed significantly more cells than did splenocytes from mice that received control injections.

Figure 21. Figure 21 shows downmodulation of human CD40L, but not murine CD40L, in lung tumor cell lines that express CD40.

Figure 22. Figure 22A shows that CD40 binding induces enhanced expression of the tumor cell surface

markers CD95 (Fas), CD54 (ICAM-1), and MHC-I, in lung tumor cell lines. Figure 22B shows downmodulation of human CD40L by CD40-positive tumor cells.

Figure 23. Figure 23 shows the inhibition of Fas ligand expression by lymphocytes in the presence of RA synovial fluid.

Figure 24. Figure 24 shows an outline for a clinical trial of an accessory molecule ligand (CD40L) gene therapy treatment for B cell CLL.

Figure 25. Figure 25 shows a sequence line-up of human Fas ligand with human Fas ligand in which Domain III is replaced by Domain III of murine Fas ligand. The top protein sequence is native human Fas ligand. Domain III is underlined with the dotted line. The double underline indicates a putative MMP cleavage site. The bottom protein sequence is that of chimeric human-mouse Fas ligand. Domain III of the mouse Fas ligand (underlined with dotted line) is substituted for Domain III of human Fas ligand. The numbers correspond to the amino acid sequence number using 1 for the start of the polypeptide sequence. The number of the first nucleotide base for the codon encoding the amino acid is  $1+3x(n-1)$ , where n is the amino acid sequence number.

Figure 26. Figure 26 shows a sequence line-up of human Fas ligand with human Fas ligand in which Domain III has been replaced with Domain III of human CD70. The top protein sequence is native human Fas ligand, and the bottom sequence is that of chimeric Fas ligand, in which Domain III of human CD70 has been substituted for Fas Domain III. Other markings are used similarly as in Figure 25.

Figure 27. Figure 27 shows a sequence line-up of human Fas ligand with human Fas ligand in which Domain I has been replaced with Domain III of human CD70. The top protein is native human Fas ligand, and the bottom

protein sequence is that of chimeric Fas ligand, in which Domain III has been replaced with Domain I of human CD70. Other markings are used similarly as in Figure 25.

- 5     Figure 28. Figure 28 shows the amino acids around and at known matrix metalloproteinase (MMP) cleavage sites, as described in Smith, M.M. et al., Journal of Biol. Chem. 270:6440-6449 (95) and Nagase, H., and G.B. Fields, Biopolymers (Peptide Science) 40:399-416 (96).  
10 The cleavage site is indicated with an arrow.

#### DETAILED DESCRIPTION OF THE INVENTION

- 15 All references cited herein are hereby incorporated in their entirety by reference.

##### I. Definitions

- 20 An "accessory molecule ligand gene" is a gene which encodes all or part of an accessory molecule ligand. The gene comprises at least the nucleotide sequence required to encode the functional portion of an accessory molecule ligand. The gene may optionally include such genetic elements as promoters, enhancers  
25 and 3' ends. The accessory molecule ligand gene is derived from a ligand which is a member of the tumor necrosis factor (TNF) family, including CD40 ligand, Fas ligand, CD70, TNF $\alpha$ , TNF $\beta$ , CD30 ligand, 4-1BB ligand (4-1BBL), nerve growth factor and TNF-related apoptosis  
30 inducing ligand (TRAIL). As used herein, the term "accessory molecule ligand gene" includes chimeric accessory molecule ligand genes as defined below.

- As used herein, the term "malignant cells or neoplastic cells," is defined to mean malignant or  
35 cancerous cells which are found in a human patient or an

animal. Preferred types of malignant or neoplastic cells include any malignant antigen-presenting cell. In some preferred embodiments, these malignant antigen presenting cells have at least low levels of CD40  
5 present on the cell surface.

As used herein, the term "neoplastic human cells" is defined to mean human cells which are neoplastic including but not limited to antigen presenting cells, any neoplastic cell which may function as an antigen  
10 presenting cell or function to facilitate antigen presentation, neoplastic monocytes, neoplastic macrophages, neoplastic B cells, neoplastic dendritic cells, neoplastic Langerhans cells, neoplastic interdigitating cells, neoplastic follicular dendritic  
15 cells, or neoplastic Kupffer cells and the like. The definition of neoplastic human cells includes those cells which are associated with neoplastic cells in the tumor bed of human patients. Typically, the neoplastic human cells are either leukemias, lymphomas, AML, ALL,  
20 AMML, CML, CMML, CLL other tumors of antigen presenting cells or breast, ovarian or lung neoplastic cells. It is also contemplated that the accessory molecule ligand genes or chimeric accessory molecule ligand genes of the present invention may be inserted into somatic cells.  
25 These somatic cells can be created by a genetic engineering process which has introduced into those cells genes which encode molecules which render those cells capable of presenting antigen to the immune system.

As used herein, the term "chimeric gene" is defined to mean a gene in which part of the gene is derived from a second different gene and combined with the first gene so that at least a portion of each gene is present in the resulting chimeric gene. A gene may be chimeric if  
35 any portion of the sequence which encodes the resulting

protein is derived from a second and different gene. Typical chimeric genes include genes in which specific functional domains from one gene have been transferred to a second gene and replace the analogous domains of that second gene. For example, the resulting chimeric gene may have one domain derived from a murine gene and several domains derived from a human gene. These domains may range in size from 5 amino acids to several hundred amino acids. Other examples of chimeric accessory molecule ligand genes include genes which contain nucleotides encoding amino acids not found in any naturally occurring accessory molecule ligand gene. Examples of chimeric genes and potential various combinations of domains are numerous and one of skill in the art will understand that no limit is placed on the amount of one gene that must be present in a second gene to render it chimeric.

As used herein, the term "murine CD40 ligand gene" is defined to mean an accessory molecule ligand gene which is derived from a murine CD40 ligand gene. Examples of such murine CD40 ligand genes include the gene isolated by Armitage et al., Nature, 357:80-82 (1992) and other genes derived from murine origin which hybridize to the gene described by Armitage et al. under low stringency hybridization conditions.

As used herein, the term "vector or genetic vector" is defined to mean a nucleic acid which is capable of replicating itself within an organism such as a bacterium or animal cell. Typical genetic vectors include the plasmids commonly used in recombinant DNA technology and various viruses capable of replicating within bacterial or animal cells. Preferred types of genetic vectors includes plasmids, phages, viruses, retroviruses, and the like.

As used herein, the term "gene therapy vector" is defined to mean a genetic vector which is capable of directly infecting cells within an animal, such as a human patient. A number of gene therapy vectors have been described in the literature, and include, the gene therapy vector described in Cantwell et al., Blood, In Press (1996) entitled "Adenovirus Vector Infection of Chronic Lymphocytic Leukemia B Cells." Such vectors have been described for example by Woll, P. J. and I. R. Hart, Ann. Oncol., 6 Suppl 1:73 (1995); Smith, K. T., A. J. Shepherd, J. E. Boyd, and G. M. Lees, Gene Ther., 3:190 (1996); Cooper, M. J., Semin. Oncol., 23:172 (1996); Shaughnessy, E., D. Lu, S. Chatterjee, and K. K. Wong, Semin. Oncol., 23:159 (1996); Glorioso, J. C., N. A. DeLuca, and D. J. Fink, Annu. Rev. Microbiol., 49:675 (1995); Flotte, T. R. and B. J. Carter, Gene Ther., 2:357 (1995); Randrianarison-Jewtougoff, V. and M. Perricaudet, Biologicals., 23:145 (1995); Kohn, D. B., Curr. Opin. Pediatr., 7:56 (1995); Vile, R. G. and S. J. Russell, Br. Med. Bull., 51:12 (1995); Russell, S. J., Semin. Cancer Biol., 5:437 (1994); and Ali, M., N. R. Lemoine, and C. J. Ring, Gene Ther., 1:367 (1994). All references cited herein are hereby incorporated by reference.

## II. Genetic Vectors and Constructs Containing an Accessory Molecule Ligand Gene

### A. Accessory Molecule Ligand Genes

In one embodiment of the present invention, preferred gene therapy vectors contain an accessory molecule ligand gene. This accessory molecule ligand gene may be derived from any source and may include molecules which are man-made and do not appear in



nature. The present invention contemplates accessory molecule ligand genes which are derived from the genes encoding molecules within the tumor necrosis family (TNF) which includes the genes encoding: murine CD40 ligand, human CD40 ligand, Fas ligand, TNF $\alpha$ , TNF $\beta$ , CD30 ligand, 4-1BB ligand, nerve growth factor, CD70, TNF-related apoptosis inducing ligand (TRAIL) and chimeric accessory molecule ligands. The nucleotide sequence of one accessory molecule ligand, the sequence of at least one form of the murine CD40 ligand gene, has been determined and is listed as SEQ ID NO: 1. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to the sequence present in SEQ ID NO: 1, and thus hybridizes to this sequence at low stringency hybridization conditions. One of skill in the art will understand that accessory molecule ligand genes, including murine CD40 ligand gene, useful in the present invention may be isolated from various different murine strains.

The nucleotide sequence of a human CD40 ligand gene has been determined and is shown as SEQ ID NO: 2. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to SEQ ID NO: 2, and thus hybridizes to this sequence at low stringency conditions. One of ordinary skill in the art will understand that the accessory molecule ligand genes, including the human CD40 ligand genes, useful in the present invention, may vary depending on the individual from which the gene is isolated and such variations may prove useful in producing unique accessory molecule ligand genes. The present invention contemplates the use of the domains, sub-domains, amino acid or nucleotide sequence of the human CD40 ligand and/or human CD40 ligand gene as part of a chimeric

accessory molecule ligand or chimeric accessory molecule ligand gene.

The nucleotide sequence of a bovine CD40 ligand gene has been determined and is shown as SEQ ID NO: 8.

5 The present invention contemplates the use of any accessory molecule ligand gene which is homologous to SEQ ID NO: 8, and thus hybridizes to the sequence at low stringency conditions. One of ordinary skill in the art will understand that the accessory molecule ligand  
10 genes, including the bovine CD40 ligand genes, may vary depending on the individual animal from which the gene is isolated and that such variations may prove useful in producing unique accessory molecule ligand genes.

The nucleotide sequence of human  $TNF_{\alpha}$  and human  $TNF_{\beta}$   
15 have been determined and are shown as SEQ ID NOS: 9 and 10, respectively. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to either human  $TNF_{\alpha}$  or human  $TNF_{\beta}$  (SEQ ID NOS: 9 and 10, respectively), and thus hybridizes to  
20 these sequences at low stringency conditions. The accessory molecule ligand genes useful in the present invention, including the human  $TNF_{\alpha}$  and  $TNF_{\beta}$  genes, may vary depending on the particular individual from which the gene has been isolated and these variations may  
25 prove useful in producing unique accessory molecule genes.

The nucleotide sequence of porcine  $TNF_{\alpha}$  and  $TNF_{\beta}$  have been determined and are shown as SEQ ID NO: 11. The present invention contemplates the use of any  
30 accessory molecule ligand gene which is homologous to either SEQ ID NO: 11, and thus would hybridize to these sequences at low stringency conditions. One of ordinary skill in the art will understand that the accessory molecule ligand genes, including the porcine  $TNF_{\alpha}$  and  
35  $TNF_{\beta}$  genes, may vary depending on the particular animal

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30 The nucleotide sequence of a human CD70 gene  
has been determined and is shown as SEQ ID NO: 15. The  
murine CD70 gene sequence has also been determined, and  
is shown as SEQ ID NO: 36 and was described by Tesselaar  
et. al, J. Immunol. 159:4959-65(1997). The present  
35 invention contemplates the use of any accessory molecule

ligand gene which is homologous to SEQ ID NO: 15 or 36, and thus hybridizes to this sequence at low stringency conditions. One of ordinary skill in the art will understand that the accessory molecule ligand genes, including the human CD70 gene may vary depending on the individual from which the gene is isolated and that these variations may prove useful in producing unique accessory molecule ligand genes.

The nucleotide sequence of human CD30 ligand gene has been determined and is shown as SEQ ID NO: 16. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to SEQ ID NO: 16, and thus hybridizes to this sequence at low stringency conditions. One of ordinary skill in the art will understand that the accessory molecule ligand genes, including the human CD30 ligand gene, may vary depending on the individual from which the gene is isolated and that such variations may prove useful in producing unique accessory molecule ligand genes.

The present invention also contemplates variations and variants of the nucleotide sequences of the accessory molecule ligand genes provided herein which are caused by alternative splicing of the messenger RNA. This alternative splicing of the messenger RNA inserts additional nucleotide sequences which may encode one or more optional amino acid segments which in turn allows the accessory molecule ligand encoded to have additional properties or functions.

The nucleotide sequence of a human and mouse 4-1BBL have been determined and are shown as SEQ ID NOS: 17 and 18, respectively. The present invention contemplates the use of any accessory molecule ligand gene which is homologous to either SEQ ID NOS: 17 or 18, and thus hybridizes to these sequences at low stringency conditions. One of ordinary skill in the art will

understand that accessory molecule ligand genes,  
including the human 4-1BBL gene may vary depending on  
the individual from which it is isolated and that such  
variations may prove useful in producing unique  
5 accessory molecule ligand genes.

The present invention also contemplates chimeric  
accessory molecules containing any domain, sub-domain  
portion, or amino acid sequence encoded by the following  
genes: bovine TNF- $\alpha$  (SEQ ID NO: 21), murine CD40 ligand  
10 (SEQ ID NO: 22), human nerve growth factor- $\beta$  (SEQ ID NO:  
23), murine nerve growth factor (SEQ ID NO: 24), rat Fas  
ligand (SEQ ID NO: 25), human TNF-related apoptosis  
inducing ligand (TRAIL) (SEQ ID NO: 41, Genbank  
accession number U37518), murine TNF-related apoptosis  
15 inducing ligand (TRAIL) (SEQ ID NO: 42, Genbank  
accession number U37522), murine CD30-Ligand (SEQ ID NO:  
43), human 4-1BBL (SEQ ID NO: 17), and murine 4-1BBL  
(SEQ ID NOS: 44 and 18). The present invention also  
contemplates chimeric accessory molecules which utilize  
20 genes encoding amino acid sequences homologous to these  
sequences.

The present invention contemplates chimeric  
accessory molecule ligand genes which are comprised of a  
nucleotide segment derived from one accessory molecule  
25 ligand gene operatively linked to a nucleotide sequence  
derived from a different accessory molecule ligand gene  
or other gene.

For example, chimeric accessory molecule ligand  
genes are contemplated which are comprised of a segment  
30 of the murine CD40 ligand gene which has been  
operatively linked to at least one other additional gene  
segment derived from a different accessory molecule  
ligand gene. The size of the particular segment derived  
from the different accessory molecule ligand gene may  
35 vary from a nucleotide sequence encoding a few amino

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acids, a sub-domain of the accessory molecule ligand, a domain of the accessory molecule ligand or more than a domain of an accessory molecule ligand. Other chimeric accessory molecules of the present invention are

5 comprised of an accessory molecule ligand gene into which nucleotides encoding an amino acid segment which is not found as part of a naturally occurring accessory molecule ligand have been inserted. This amino acid segment may be artificially created or derived from a

10 protein found in nature. The chimeric accessory molecule ligand gene encodes a chimeric amino acid sequence and thus a chimeric accessory molecule ligand encoded may possess unique properties in addition to the properties found on the individual segments derived from

15 the different accessory molecule ligand genes. The chimeric accessory molecule ligand gene may encode an accessory molecule ligand which has properties derived from the accessory molecule ligand used to construct the chimeric gene.

20 Each of the accessory molecule ligand genes which are a member of the tumor necrosis factor family have a similar secondary structure consisting of a number of domains. This domain structure includes a first domain which is encoded by the 5' region of the accessory

25 molecule ligand gene. The second domain (Domain II) is the domain which contains the amino acids which span the cell membrane and is thus called the transmembrane domain. The third domain (Domain III) is the proximal extracellular domain and these amino acids are the amino

30 acids which are found proximal to the cellular membrane. The fourth domain (Domain IV), is encoded by the 3' end of the accessory molecule ligand gene and has been called the distal extracellular domain. The distal extracellular domain (Domain IV) generally makes up the

35 soluble form of the tumor necrosis factor family

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molecule. Based on the x-ray crystal structure of human TNF, the predicted secondary structure of the accessory molecule, CD40 ligand has been deduced together with the domain structure of these molecules by M. Peitsch and C. Jongeneel, International Immunology, 5:233-238 (1993). The secondary structures of the other members of the tumor necrosis factor family were deduced using computer analysis together with comparison to the human TNF and CD40 ligand domain structure. In Table I, the domain boundaries of a number of accessory molecule ligand genes is shown. A diagram of these domains for a number of these accessory cell molecule ligands is shown in Figure 1. The assignments of the domain boundaries are approximate and one of ordinary skill in the art will understand that these boundaries may vary and yet still provide useful identification of domains.

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		Domain I (Cytoplasmic)	Domain II (Transmembrane)	Domain III (Proximal Extracellular)	Domain IV (Distal Extracellular)
5	Human CD40 Ligand	1-42	43-135	136-330	331-786
	Murine CD40 Ligand	1-42	43-135	136-327	328-783
10	Bovine CD40 Ligand	1-42	43-135	136-330	331-786
	Human TNF- $\alpha$	1-87	88-168	169-228	229-699
	Murine TNF- $\alpha$	1-87	88-168	169-237	238-705
	Porcine TNF- $\alpha$	1-87	88-168	169-228	229-696
	Human TNF- $\beta$	1-39	40-129	130-153	154-615
15	Porcine TNF- $\beta$	1-39	40-126	127-150	151-612
	Human Fas Ligand	1-237	238-315	316-390	391-843
	Murine Fas Ligand	1-237	238-309	310-384	385-837
20	Human CD70	1-61	62-117	118-132	133-579
	Murine CD70	1-73	74-123	124-138	139-585
	Human CD30 Ligand	1-117	118-186	187-240	241-702
25	Murine CD30 Ligand	1-135	136-201	202-255	256-717
	Human 4-1BBL	1-69	70-174	175-210	211-762
30	Murine 4-1BBL	1-237	238-333	334-369	370-927
	Human TRAIL	1-39	40-117	118-375	376-843
35	Murine TRAIL	1-51	52-111	112-387	388-873

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One of ordinary skill in the art will understand that typical chimeric accessory molecule genes would include genes produced by exchanging domains or sub-domain segments between, for example, a mouse CD40  
5 ligand gene and a human CD40 ligand gene. For example, chimeric accessory molecule gene may be constructed by operatively linking Domain I of the human CD40 ligand gene to Domains II-IV of the murine CD40 ligand gene. One of ordinary skill in the art will understand the  
10 variety of chimeric accessory molecule ligand genes which may be produced using the accessory molecules identified in Table I. The present invention also contemplates chimeric accessory molecules which are not shown in Table I but which are shown to have a similar  
15 domain structure. Other chimeric genes are also contemplated in which smaller segments (sub-domain segments) are exchanged between, for example, a murine CD40 ligand gene and a human CD40 ligand gene or a second murine CD40 ligand gene. One of skill in the art  
20 will understand that genes encoding accessory molecules will have at least gene segments which correspond to various functional segments of an accessory molecule ligand such as the murine CD40 ligand encoded by the murine CD40 ligand gene (SEQ ID NO: <sup>3</sup>1). It will also be  
25 apparent to one of skill in the art that the nucleotide boundaries identified in Table I may vary considerably from those identified for the murine CD40 ligand gene (SEQ ID NO: <sup>2</sup>1) and still define domains which are useful in the present invention.

30 In one preferred embodiment, the chimeric accessory molecule ligand gene is comprised of the nucleotides encoding extracellular domains (Domains III and IV) of human CD40 ligand operatively linked to the nucleotides encoding transmembrane (Domain II) and the nucleotides  
35 encoding cytoplasmic domain (Domain I) of the murine

CD40 ligand gene. Examples of such preferred chimeric accessory molecules are shown in Figure 2. An exemplary nucleotide sequence for such a gene is SEQ ID NO: 7. In other chimeric accessory molecule ligand genes of the present invention, the nucleotides encoding the extracellular domains (Domains III and IV) of the murine CD40 ligand gene may be operatively linked to nucleotides encoding the transmembrane (Domain II) and cytoplasmic domain (Domain I) of the human CD40 ligand gene. An exemplary nucleotide sequence for such a gene is SEQ ID NO: 3. In other preferred chimeric accessory molecule ligand genes of the present invention, the nucleotides encoding the extracellular domains (Domains III and IV) and transmembrane domain (Domain II) of human CD40 ligand are coupled to the nucleotides encoding cytoplasmic domain (Domain I) of murine CD40 ligand gene. An exemplary nucleotide sequence for such a gene is SEQ ID NO: 6. Other chimeric accessory molecule genes contemplated by the present invention comprise the nucleotides encoding the extracellular domains (Domains III and IV) and transmembrane domain (Domain II) of the murine CD40 ligand gene operatively linked to the nucleotides encoding cytoplasmic domain of the human CD40 ligand gene. An exemplary nucleotide sequence for such a gene is SEQ ID NO: 5. Other chimeric accessory molecule ligand genes are contemplated by the present invention in which the human CD40 ligand gene extracellular domains (Domain III and IV) is operatively linked to the murine CD40 ligand gene transmembrane domain (Domain II) which is operatively linked to the human CD40 ligand gene cytoplasmic domain (Domain I). An exemplary nucleotide sequence for such a gene is SEQ ID NO: 4.

One of ordinary skill in the art will understand that many more combinations which utilize domains or

other selected segments of any of the accessory molecule ligand genes including the human CD40 ligand genes and the mouse CD40 ligand genes are possible. Such additional chimeric accessory molecule genes would

5 include the following genes: chimeric accessory molecule genes in which the nucleotides encoding Domain I are selected from a particular accessory molecule ligand gene and operatively linked, either directly or by an additional nucleotide sequence to the nucleotides

10 encoding Domain II from a particular accessory molecule ligand gene. These domains then would be operatively linked either directly or by an additional nucleotide sequence to the nucleotides encoding Domain III from a particular accessory molecule ligand gene. This

15 molecule would then be operatively linked either directly or by an additional nucleotide sequence to the nucleotides encoding Domain IV of a particular accessory molecule ligand gene. The chimeric accessory molecule ligand gene constructed in this manner may have

20 additional nucleotides on either end or between domains which are useful to provide different amino acids in these positions. One of ordinary skill in the art will understand that these particular combinations are merely illustrations and that numerous other combinations could

25 be contemplated in which gene segments comprising nucleotides encoding less than the entire domain of an accessory molecule are exchanged between different accessory molecules.

The present invention also contemplates chimeric

30 accessory molecule ligand genes which are comprised of gene segments of mouse or human CD40 ligand in combination with gene segments derived from Fas ligand,  $TNF_{\alpha}$ ,  $TNF_{\beta}$ , CD70, CD30L, 4-1BBL, nerve growth factor or TNF-related apoptosis inducing ligand (TRAIL).

35 Particularly useful chimeric accessory molecule ligand

genes comprise at least one gene segment which is derived from a murine CD40 ligand gene together with gene segments or a gene segments derived from a different accessory molecule ligand gene.

5       The present invention also contemplates chimeric accessory molecule ligand genes in which the accessory molecules produced have been modified to remove amino acids within the chimeric accessory molecule that are used by post-translational mechanisms to regulate the  
10 level of expression of the accessory molecule or accessory molecule protein on a particular cell. The sites removed from the chimeric accessory molecules or chimeric molecule may include amino acids or sites which make up protease cleavage sites including  
15 metallothionine proteases, serine proteases and other proteases that recognize an amino acid sequence either specifically or nonspecifically. In particular preferred embodiments, amino acids in Domain III which make up potential or actual recognition site(s) used by  
20 post-translational regulatory mechanisms have been modified or removed.

      The present invention also contemplates chimeric accessory molecule ligand genes in which the domains, subdomain fragments or other amino acid residues have  
25 been taken from one accessory molecule ligand gene and moved into a second accessory molecule ligand gene from the same species. For example, in this particular embodiment, the human Domain I, and the human Domain II from the CD40 ligand molecule may be operatively linked  
30 to the nucleotides encoding the human Domain III from, for example, the CD70 molecule which is in turn operatively linked to human Domain IV for the CD40 ligand molecule. This chimeric accessory molecule therefore contains human CD40L Domains I, II and IV and  
35 human CD70 Domain III. An exemplary nucleotide sequence

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[illegible]

of sub-domain nucleotide segments allows the introduction of short amino acid sequences derived from other molecules into chimeric accessory molecules of the present invention. The incorporation of such short sub-domain segments or amino acid changes into the accessory molecule ligand allows the introduction of desired or the removal of undesired features of that molecule.

The identification of domain structures within accessory cell molecules is well known in the art and generally requires the identification of cysteine residues within the accessory molecules and the subsequent mapping of disulfide bonds between various cysteine residues. The mapping of various sub-domain segments of an accessory molecule is well known in the art and involves analysis of the amino acid sequence of the accessory molecules and generally involves a comparison of the crystal structure of tissue necrosis factor with the use of predictive algorithms thereby producing a predicted structure of a chimeric accessory molecule or an accessory molecule. This predicted structure of these molecules can then be used to select various sub-domain portions of the molecule to be used to construct further chimeric accessory molecules. Examples of such mapping studies include the studies by M. Pitsch and C. V. Jongeneel, International Immunology, 5:233-238 (1993) and the analysis shown in Figure 1.

The present invention also contemplates accessory molecule ligand genes and chimeric accessory molecule ligand genes which are truncated and encode less than the full length of the amino acid sequence found in the native accessory molecule ligand. These truncations may alter the properties of the accessory molecule ligand gene but some identified activity is maintained. Such truncations may be made by removing a gene segment or gene segments from the accessory molecule gene and

typically would be performed by removing nucleotides encoding domains which are not directly involved in the binding of the accessory molecule ligand with its accessory molecule. These truncated accessory molecule  
5 ligand genes or chimeric truncated accessory molecule ligand genes may contain further gene segments which encode amino acid segments or domains which replace the domains removed from that truncated accessory molecule gene. However, such replacement of the portions of the  
10 accessory molecule removed by truncation is not necessary.

The chimeric accessory molecule genes of the present invention may be constructed using standard genetic engineering methods to operatively link a  
15 particular nucleotide sequence from one accessory molecule ligand gene to a different nucleotide sequence derived from the same or different accessory molecule ligand gene. In addition, standard genetic engineering methods may be used to insert man-made nucleotide  
20 sequences or sub-domain nucleotide sequences into the chimeric accessory molecule ligand gene. One of ordinary skill in the art will understand that various methods may be utilized to produce such chimeric accessory molecule genes. For example, a gene  
25 conversion method known as "SOEN" may be used to produce a chimeric accessory molecule gene which contains nucleotide segments derived from different chimeric accessory molecules. The methods for using this gene conversion method are well known in the art and have  
30 been described for example in Horton, R. M., Mol. Biotechnol., 3:93 (1995); Ali, S. A. and A. Steinkasserer, Biotechniques, 18:746 (1995); Vilardaga, J. P., E. Di Paolo, and A. Bollen, Biotechniques, 18:604 (1995); Majumder, K., F. A. Fattah, A. Selvapandiyan,  
35 and R. K. Bhatnagar, PCR. Methods Appl., 4:212 (1995);

Boles, E. and T. Miosga, Curr. Genet. 28:197 (1995);  
Vallejo, A. N., R. J. Pogulis, and L. R. Pease, PCR.  
Methods Appl., 4:S123 (1994); Henkel, T. and P. A.  
Baeuerle, Anal. Biochem., 214:351 (1993); Tessier, D. C.  
5 and D. Y. Thomas, Biotechniques, 15:498 (1993);  
Morrison, H. G. and R. C. Desrosiers, Biotechniques,  
14:454 (1993); Cadwell, R. C. and G. F. Joyce, PCR.  
Methods Appl., 2:28 (1992); and, Stappert, J., J.  
Wirsching, and R. Kemler, Nucleic Acids Res., 20:624  
10 (1992). Alternatively, one of ordinary skill in the art  
will understand that site-directed mutagenesis may be  
used to introduce changes into a particular nucleotide  
sequence to directly produce or indirectly be used to  
produce a chimeric accessory molecule gene of the  
15 present invention. For example, the mutagen kit  
provided by BioRad Laboratories may be used together  
with the methods and protocols described within that kit  
to produce the desired changes in the nucleotide  
sequence. These methods were originally described by  
20 Kunkel, Proc. Natl. Acad. Sci. USA, 82:488-492 (1985)  
and Kunkel et al., Meth. Enzol. Mol., 154:367-382  
(1987). By using the site directed mutagenesis protocols  
described herein and known within the art, a skilled  
investigator may induce individual nucleotide changes  
25 which result in an altered amino acid sequence or which  
preserve an amino acid sequence but introduce a desired  
restriction enzyme recognition sequence into the gene.  
This new restriction endonuclease recognition site may  
then be used to cut the gene at that particular point  
30 and use it to a gene or segment of another accessory  
molecule ligand gene. In addition to these methods, one  
of ordinary skill in the art will understand that an  
entire chimeric accessory molecule ligand gene may be  
synthesized using synthetic methods known in the art.  
35 This methodology only requires that the skilled artisan



generating nucleotide sequence of a chimeric accessory molecule ligand gene and provide that sequence to a company which is capable of synthesizing such a gene.

5        B.    Genetic Constructs

10        The present invention contemplates the use of accessory molecule ligand genes or chimeric accessory molecule ligand genes which are present in various types of genetic vectors. A genetic vector refers to a DNA molecule capable of autonomous replication in a cell into which another DNA segment can be inserted to cause the additional DNA segments to replicate. Vectors capable of expressing genes contained in that vector are referred to as "expression vectors." Thus, the genetic vectors and expression vectors of the present invention are recombinant DNA molecules which comprise at least two nucleotide sequences not normally found together in nature.

20        The genetic vectors useful in the present invention contain an accessory molecule ligand gene which encodes an accessory molecule ligand which is optionally operatively linked to a suitable transcriptional or translational regulatory nucleotide sequence, such as one derived from a mammalian, microbial, viral, or insect gene. Such regulatory sequences include sequences having a regulatory role in gene expression, such as a transcriptional promoter or enhancer, an operator sequence to control transcription, a sequence encoding a ribosomal binding site within the messenger RNA and appropriate sequences which control transcription, translation initiation or transcription termination.

30        Particularly useful regulatory sequences include the promoter regions from various mammalian, viral,

microbial, and insect genes. The promoter region directs an initiation of transcription of the gene and causes transcription of DNA through and including the accessory molecule ligand gene. Useful promoter regions include the promoter found in the Rous Sarcoma Virus (RSV) - long terminal repeat (LTR), human cytomegalovirus (HCMV) enhancer/promoter region lac promoters, and promoters isolated from adenovirus, and any other promoter known by one of ordinary skill in the art would understand to be useful for gene expression in eukaryotes, prokaryotes, viruses, or microbial cells. Other promoters that are particularly useful for expressing genes and proteins within eukaryotic cells include mammalian cell promoter sequences and enhancer sequences such as those derived from polyoma virus, adenovirus, simian virus 40 (SV40), and the human cytomegalovirus. Particularly useful are the viral early and late promoters which are typically found adjacent to the viral origin of replication in viruses such as the SV40. Examples of various promoters which have been used in expression vectors have been described by Okima and Berg (Mol. Cell. Biol. 3:280, 1983), the pMLSVN SV40 described by Kossman et al., Nature 312:768 (1984). One of ordinary skill in the art will understand that the selection of a particular useful promoter depends on the exact cell lines and the other various parameters of the genetic construct to be used to express the accessory molecule ligand gene or the chimeric accessory molecule ligand gene within a particular cell line. In addition, one of ordinary skill in the art will select a promoter which is known to express genes in the target cell at a sufficiently high level to be useful in the present invention.

The genetic vectors and expression vectors of the present invention optionally contain various additional

regulatory sequences including ribosome binding sites which allow the efficient translation of the messenger RNA produced from an expression vector into proteins, the DNA sequence encoding various signals peptides which may be operatively linked to the accessory molecule ligand gene or the chimeric accessory molecule ligand gene. The signal peptide, if present, is expressed as a precursor amino acid which enables improved extracellular secretion of translation fusion polypeptide.

The genetic constructs contemplated by the present invention therefore include various forms of accessory molecule ligand genes described above which are operatively linked to either a promoter sequence or a promoter and enhancer sequence and also operatively linked to a polyadenylation sequence which directs the termination and polyadenylation of messenger RNA. It is also contemplated that the genetic constructs of the present invention will contain other genetic sequences which allow for the efficient replication and expression of that construct within the desired cells. Such sequence may include introns which are derived from native accessory molecule ligand genes or, for example, from a virus gene.

The present invention also contemplates gene therapy vectors which are able to directly infect mammalian cells so as to introduce the desired accessory molecule ligand gene or chimeric accessory molecule ligand gene into that cell. These gene therapy vectors are useful for directly infecting cells which have been isolated from an animal or patient, or can be directly introduced into an animal or patient and thereby directly infect the desired cell within that animal or patient.

Many types of gene therapy vectors which are able to successfully transfer genes and cause the expression of desired foreign DNA sequences have been developed and described in the literature. For example, the article  
5 entitled "Gene Transfer Vectors for Mammalian Cells" in Current Comm. Mol. Biol., Cold Springs Harbor Laboratory, New York (1987). Further, naked DNA can be physically introduced into eukaryotic cells including human cells by transvection using any number of  
10 techniques including calcium phosphate transfection (Berman et al., Proc. Natl. Acad. Sci. USA, 81:7176 (1984)), DEAE-Dextran Transfection, protoplast fusion (Deans et al., Proc. Natl. Acad. Sci. USA, 81:1292 (1984)), electroporation, liposome fusion, polybrene  
15 transfection and direct gene transfer by laser micropuncture of the cell membrane. In addition, one of ordinary skill in the art will understand that any technique which is able to successfully introduce the DNA into a cell in such a manner as to allow it to  
20 integrate into the genome of a cell and allow the expression of the desired gene would be useful in the present invention.

Specifically, gene therapy vectors which utilize recombinant infectious virus particles for gene delivery  
25 have been widely described. See, for example, Brody, S. L. and R. G. Crystal, Ann. N. Y. Acad. Sci., 716:90 (1994); Srivastava, A., Blood. Cells, 20:531 (1994); Jolly, D., Cancer Gene Ther., 1:51 (1994); Russell, S. J., Eur. J. Cancer, 30A:1165 (1994); Yee, J. K., T.  
30 Friedmann, and J. C. Burns, Methods Cell Biol., 43 Pt A:99 (1994); Boris-Lawrie, K. A. and H. M. Temin, Curr. Opin. Genet. Dev., 3:102 (1993); Tolstoshev, P., Annu. Rev. Pharmacol. Toxicol., 33:573 (1993); and, Carter, B. J., Curr. Opin. Biotechnol., 3:533 (1992). The present  
35 invention contemplates the use of gene therapy vectors

to carry out the desired methodology of the present invention by introducing a gene encoding an accessory molecule ligand gene or a chimeric accessory molecule ligand gene into the cell. Many viral vectors have been  
5 defined and used as gene therapy vectors and include virus vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated viruses, and retroviruses. One of ordinary skill in the art will understand that useful gene therapy vectors are vectors  
10 which are able to directly introduce into the target cells the DNA which encodes the accessory molecule ligand and allow that DNA to persist in the cell so as to express the accessory molecule ligand in the desired manner within the cell.

15 The gene therapy vectors of the present invention are useful for introducing accessory molecule ligand genes into a variety of mammalian cells including human cells. The particular cells infected by the gene therapy vector will depend on the various specifics of  
20 the vector and such vectors can be used to introduce the accessory molecule ligand genes of the present invention into hematopoietic or lymphoid stem cells, antigen presenting cells, embryonic stem cells, and other cells which are capable of presenting antigen within the  
25 immune system including cells which have CD40 on their surface. Further, such gene therapy vectors are able to introduce a gene encoding an accessory molecule ligand gene into a human neoplastic cell such as a lymphoma, leukemia, AML, CLL, CML, AMML, CMML, breast cancer, lung  
30 cancer, ovarian cancer or any tumor capable of acting as antigen presenting cells or cells which can stimulate bystander antigen presenting cells. Further, the contemplated gene therapy vectors may be used to introduce the accessory molecule ligand genes of the  
35 present invention into cells which have been engineered

to make those cells capable of presenting antigen to the immune system.

5     III. Cells Containing Genetic Constructs Encoding an Accessory Molecule Ligand or Chimeric Accessory Molecule Ligand

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      The present invention also contemplates various cells which contain the genetic constructs of the present invention. These cells contain the constructs which encode the accessory molecule ligand gene and thus contain the various genetic elements described in Section II.B. above. These cells may be microbial cells, eukaryotic cells, insect cells, and various mammalian cells including human cells. In preferred embodiments of the present invention, these cells include various neoplastic cells including human neoplastic cells. These neoplastic cells may be of any cell type and include cells of the immune system, and other blood cells. Particularly preferred are any neoplastic cells which may function as an antigen presenting cells within the immune system or which may stimulate bystander antigen presenting cells by expression of a transgenic accessory cell molecule of the present invention. Typically these neoplastic which are able to function to present antigen to the immune system have or have had an accessory molecule, such as the CD40 molecule, on the cell surface. Generally, these cells are naturally capable of presenting antigen to the immune system, but the present invention also contemplates the introduction of accessory molecule ligand genes into a cell which is not naturally able to present antigen to the immune system but which has been genetically engineered to make that cell capable of presenting antigen to the immune system. Typically, these cells include various known cell types such as

monocytes, macrophages, B cells, Langerhans cells, interdigitating cells, follicular dendritic cells or Kupffer cells and the like which have become neoplastic. In addition, the present invention also contemplates  
5 cells from various carcinomas, breast, ovarian and lung cancers which contain the genetic constructs described herein. In other preferred embodiments, an accessory molecule ligand gene of the present invention is placed into cells which may be injected into a treatment site  
10 such as a tumor bed or joint. For example, the accessory molecule ligand gene of the present invention may be inserted into a fibroblast cell and the accessory molecule ligand expressed on the surface of that cell. The fibroblasts are then injected into the treatment  
15 site and cause the desired immuno effect due to the presence of the accessory molecule ligand on the surface of those cells. These cells stimulate other immune cells present in that treatment site (bystander cells). This process then results in the desired effect on the  
20 immune system.

#### IV. Methods Utilizing Genetic Vectors and Constructs Containing an Accessory Molecule Ligand Gene

25 The present invention contemplates methods of altering the immunoreactivity of human cells using a method which includes introducing a gene encoding an accessory molecule ligand gene into the human cells so that the accessory molecule ligand encoded by that gene  
30 is expressed on the surface of those cells. The present invention is useful for any human cells which participate in an immune reaction either as a target for the immune system or as part of the immune system which responds to the foreign target. A large variety of  
35 methods are contemplated in which the final result is that the accessory molecule ligand gene is introduced

into the desired cells. These methods include ex vivo methods, in vivo methods and various other methods which involve injection of DNA, genetic vectors or gene therapy vectors into the animal or human, including  
5 injection directly into the tumor bed present in any animal or human.

Ex vivo methods are contemplated wherein the cells into which the accessory molecule ligand gene is to be introduced are isolated from the animal or patient and  
10 then the gene is introduced into those isolated cells using suitable methods. Examples of useful ex vivo methods have been described for example by Raper, S. E., M. Grossman, D. J. Rader, J. G. Thoene, B. J. Clark, D. M. Kolansky, D. W. Muller, and J. M. Wilson, Ann. Surg.,  
15 223:116 (1996); Lu, L., R. N. Shen, and H. E. Broxmeyer, Crit. Rev. Oncol. Hematol., 22:61 (1996); Koc, O. N., J. A. Allay, K. Lee, B. M. Davis, J. S. Reese, and S. L. Gerson, Semin. Oncol., 23:46 (1996); Fisher, L. J. and J. Ray, Curr. Opin. Neurobiol., 4:735 (1994); and,  
20 Goldspiel, B. R., L. Green, and K. A. Calis, Clin. Pharm., 12:488 (1993). D. Dilloo et al., in Blood 90:1927-1933 (1997), describe a method, using CD40L-activated cells, for treating B-acute lymphoblastic leukemia (ALL). They cocultured leukemia cells with  
25 fibroblasts infected with a retroviral vector encoding CD40L, then injected the cell mix into mice. Such an approach, if taken in humans, would differ from that contemplated here in that the therapeutic cells are stimulated in vitro, by another cell line expressing the  
30 accessory molecule ligand. Schultze, J.L. et al., in Blood 89: 3806-3816 (1997), describe a method for stimulating T-TILs (tumor-infiltrating T cells) cytotoxic for follicular lymphoma (FL) cells by exposing them, in vitro, to FL B cells which were previously  
35 cultured with CD40L-expressing fibroblasts. They



propose an adoptive immunotherapy in which T-TILS  
stimulated in this manner are transfused into patients.  
This method also requires in vitro stimulation, of the  
cells to be transfused, with another cell line  
5 expressing an accessory molecule.

Following the introduction of the gene, including  
any optional steps to assure that the accessory molecule  
ligand gene has been successfully introduced into those  
isolated cells, the isolated cells are introduced into  
10 the patient either at a specific site or directly into  
the circulation of the patient. In preferred  
embodiments of the present invention, cell surface  
markers, including molecules such tumor markers or  
antigens identify the cells are used to specifically  
15 isolate these molecules from the patient. One of  
ordinary skill in the art will understand that such  
isolation methods are well known and include such  
methodologies as fluorescence activated cell sorting  
(FACS), immunoselection involving a variety of formats  
20 including panning, columns and other similar methods.

The present invention also contemplates introducing  
the accessory molecule ligand gene into the desired  
cells within the body of an animal or human patient  
without first removing those cells from the patient.  
25 Methods for introducing genes into specific cells in  
vivo, or within the patient's body are well known and  
include use of gene therapy vectors and direct injection  
of various genetic constructs into the animal or  
patient. Examples of useful methods have been described  
30 by Danko, I. and J. A. Wolff, Vaccine, 12:1499 (1994);  
Raz, E., A. Watanabe, S. M. Baird, R. A. Eisenberg, T.  
B. Parr, M. Lotz, T. J. Kipps, and D. A. Carson, Proc.  
Natl. Acad. Sci. U. S. A., 90:4523 (1993); Davis, H. L.,  
R. G. Whalen, and B. A. Demeneix, Hum. Gene Ther., 4:151  
35 (1993); Sugaya, S., K. Fujita, A. Kikuchi, H. Ueda, K.

[illegible]

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or directly into the tumor bed of the animal or patient. Alternatively, DNA from a genetic construct containing at least an accessory molecule ligand gene is used and directly injected into the animal.

5 In preferred embodiments of the present invention, the immune reaction or response of a human patient or animal is altered by introducing the accessory molecule ligand gene into cells, including human cells which have an accessory molecule present on the cell surface. Such  
10 cells include human cells, human antigen presenting cells and optionally these cells may be neoplastic antigen presenting cells which have the capacity to express the accessory molecule on the surface of the cell or cells which are capable of stimulating. In some  
15 embodiments, the amount of accessory molecule present on the surface of the cells into which the accessory molecule ligand gene is to be introduced is very small and such small amounts of the accessory molecule may result from down-regulation of that accessory molecule  
20 on the surface of such cells. In some embodiments, the cells into which the accessory molecule ligand gene is introduced have at least low levels of the CD40 molecule present on the cell surface or are derived from cells which did express the CD40 ligand molecule on the cell  
25 surface but have reduced or eliminated that expression.

The preferred methods of altering the immunoreactivity of a particular cell are applicable to mammalian cells including human cells. These human cells may include neoplastic human cells such as human  
30 lymphomas, leukemias, and other malignancies including breast, lung and ovarian cancers. In some preferred embodiments the cells are normal antigen presenting cells of a human patient such as monocytes, macrophages, B cells, Langerhans cells, interdigitating cells,  
35 follicular dendritic cells, Kupffer cells, and other

similar cells. In preferred embodiments, the cells are lymphocytes which acquire altered immunoreactivity when the accessory molecules of the present invention are introduced into those cells. In other preferred

5   embodiments, the cells may be neoplastic or normal cells which are capable of stimulating bystander antigen presenting cells when the accessory molecule ligand genes of the present invention are introduced into these cells. The present invention also contemplates that

10   cells which are not naturally capable of presenting antigen to the immune system may be genetically engineered to introduce the genes encoding the molecules required for antigen presentation, including genes encoding an accessory molecule, and thus allow these

15   cells to act as artificial antigen presenting cells. The accessory molecule ligand gene may then be introduced into these artificial antigen presenting cells. Various tests are well known in the literature to determine whether a particular cell is able to

20   function as an antigen presenting cell, such as cell proliferation or the production of lymphokines and therefore this aspect of the present invention may be easily determined.

In addition to the above normal human cells, the

25   present invention also contemplates introducing the accessory molecule ligand gene into various neoplastic or malignant cells which optionally are antigen presenting cells. Such human neoplastic cells which are contemplated include leukemias, lymphomas, AML, AMML, or

30   CMML, CML, CLL and any neoplastic cell which is capable of stimulating bystander antigen presenting cells when an accessory molecule ligand is introduced into that cell. Also contemplated are neoplastic cells such as a breast, ovarian or lung cancer cell which is capable of

35   or is engineered to act as an antigen presenting cell.

However, the present immunomodulation also applicable to other malignancies not specifically identified and thus would include any tumor of any cell capable of presenting antigen within the animal or human immune system or any cell which is capable of acting as an antigen presenting cell or capable of stimulating bystanding antigen presenting cells after an accessory molecule ligand gene has been introduced into those cells. Generally these antigen presenting cells have accessory molecules on the surface of the cells.

The present methods of altering the immunoreactivity of a human or animal cell contemplate the introduction of an accessory molecule ligand gene into the cells for which altered immunoreactivity is desired. The genes useful in the present invention include the wide range of accessory molecule ligand genes and chimeric accessory molecule ligand genes identified above and in preferred embodiments include at least a portion of the murine CD40 ligand gene. In particularly preferred embodiments, the accessory molecule ligand gene introduced into the cells using the methods of the present invention is selected to correspond to the accessory molecule present on the surface of the cells for which altered immunoreactivity is desired. In one particular application of the present invention, the immunoreactivity of a cell which expresses the CD40 molecule on the cell surface would be accomplished by introducing the gene which encodes the CD40 ligand molecule and more preferably the murine CD40 ligand molecule.

The present invention also contemplates altering the immunoreactivity of human or animal cells by introducing an accessory molecule ligand gene which is a chimeric accessory molecule ligand gene into the cell. The various useful chimeric accessory molecule ligand

genes were identified above and could include a wide variety of molecules and allow the unique properties of those chimeric accessory molecule ligand genes to be utilized to alter the immunoreactivity of the target  
5 cells. In preferred embodiments, useful chimeric accessory molecule ligand genes are genes which encode at least a portion of the accessory molecule ligand which is capable of binding the accessory molecule present on the surface of the cells for which altered  
10 immunoreactivity is desired.

The methods of the present invention for altering the immunoreactivity contemplate the use of genetic vectors and genetic constructs including gene therapy vectors which encode an accessory molecule ligand and  
15 therefore contain an accessory molecule ligand gene. Typically, the genetic vectors and genetic constructs including the gene therapy vectors of the present invention have a promoter which is operatively linked to the accessory molecule ligand gene followed by a  
20 polyadenylation sequence. In other embodiments, the only requirement is that the genetic vectors, genetic constructs, and gene therapy vectors of the present invention contain the accessory molecule ligand gene or the chimeric accessory molecule ligand gene.

25

#### V. Methods of Treating Neoplasia

The present invention also contemplates methods of treating human neoplasia comprising inserting into a  
30 human neoplastic cell a gene which encodes an accessory molecule ligand so that the accessory molecule ligand is expressed on the surface of the neoplastic cells. The present invention contemplates treating human neoplasia both in vivo, ex vivo and by directly injecting various  
35 DNA molecules containing a gene which encodes an

accessory molecule ligand into the patient. However, at a minimum, the present methods for treating human neoplasia involve inserting the gene encoding the accessory molecule ligand into the neoplastic cells in such a way as to allow those neoplastic cells to express the accessory molecule ligand on the cell surface. The expression of the accessory molecule ligand gene in these neoplastic cells modulates the immune system to cause the neoplasia to be reduced or eliminated.

In a preferred method of treating human neoplasia, the method further comprises the steps of first obtaining the human neoplastic cells from a human patient and then inserting into the isolated human neoplastic cells a gene which encodes an accessory molecule ligand so that the accessory molecule ligand is expressed on the surface of the neoplastic cells. The human neoplastic cells having the accessory molecule ligand on the surface of that cell are then infused back into the human patient. One of ordinary skill in the art will understand that numerous methods are applicable for infusing the altered human neoplastic cells containing the gene encoding the accessory molecule ligand back into the patient and that these methods are well known in the art.

The contemplated methods of treating human neoplasia are applicable to a wide variety of human neoplasias including lymphomas, leukemias, and other malignancies. In preferred embodiments the human neoplasia is a neoplasia which involves the antigen presenting cells of the human immune system and includes monocytes, macrophages, B cells, Langerhans cells, interdigitating cells, follicular dendritic cells, Kupffer cells, and the like. In other preferred embodiments, the human neoplasia is a leukemia, a lymphoma, AML, AMML, CMML, CML or CLL, lung cancer,

breast cancer, ovarian cancer and other similar neoplasias.

The genetic vectors, genetic constructs and gene therapy vectors useful in the methods of treating human neoplasia of the present invention have been disclosed above and include constructs in which a promoter is operatively linked to the accessory molecule ligand gene or the chimeric accessory molecule ligand gene which is in turn operatively linked to a polyadenylation sequence. The methods of treating human neoplasia contemplate the use of genetic constructs, genetic vectors and gene therapy vectors as described in this specification. In addition, the present invention contemplates the use of DNA which contains at least a gene encoding an accessory molecule ligand gene. This gene may or may not contain a promoter and other regulatory sequences.

In preferred embodiments of the present invention, the cells comprising the human neoplasia are located in at least one defined site termed a tumor bed within the human patient. This tumor bed typically contains the tumor or neoplastic cell together with a number of other cells which are associated with the tumor or neoplastic cells. The present invention contemplates methods of treating such human neoplasia present in a tumor bed by injecting into the tumor bed of the patient, a gene which encodes an accessory molecule ligand so that the accessory molecule ligand is expressed on the surface of the tumor cells thereby causing the cells to participate in an immune reaction. The gene which encodes the accessory molecule ligand may be present as part of a gene therapy vector, genetic construct or genetic vector.

In preferred embodiments, the accessory molecule ligand gene is a chimeric accessory molecule ligand gene



which has at least a portion of the murine CD40 ligand gene is used. In other preferred embodiments, the accessory molecule ligand encoded is capable of binding an accessory molecule present on the human neoplasia to be treated.

The various gene therapy vectors used in the treatment methods of the present invention include vectors which are capable of directly infecting human cells. Such vectors have been described in the literature and are readily adaptable to the methods described in the present invention.

The present invention contemplates the use of any type of gene therapy including the methods of Raper, S.E. et al., Ann. Surg., 223:116 (1996); Lu, L. et al., Crit. Rev. Oncol. Hematol., 22:61 (1996); Koc, O. N. et al., Semin. Oncol., 23:46 (1996); Fisher, L. J. et al., Curr. Opin. Neurobiol., 4:735 (1994); Goldspiel, B. R. et al., Clin. Pharm., 12:488 (1993); Danko, I. et al., Vaccine, 12:1499 (1994); Raz, E. et al., Proc. Natl. Acad. Sci. U.S.A., 90:4523 (1993); Davis, H. L. et al., Hum. Gene Ther., 4:151 (1993); Sugaya, S. et al., Hum. Gene Ther., 7:223 (1996); Prentice, H. et al., J. Mol. Cell Cardiol., 28:133 (1996); Soubrane, C. et al., Eur. J. Cancer, 32A:691 (1996); Kass-Eisler, A. et al., ann. N. Y. Acad. Sci., 772:232 (1995); DeMatteo, R. P. et al., Ann. Surg., 222:229 (1995); Addison, C. L. et al., Proc. Natl. Acad. Sci. U.S.A., 92:8522 (1995); Hengge, U. R. et al., J. Clin. Invest., 97:2911 (1996); Felgner, P. L. et al., Ann. N. Y. Acad. Sci., 772:126 (1995); Furth, P.A., Hybridoma, 14:149 (1995); Yovandich, J. et al., Hum. Gene Ther., 6:603 (1995); Evans, C.H. et al., Hum. Gene Ther., 7:1261.

#### VI. Methods of Vaccination

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The present invention contemplates methods of vaccinating an animal against a predetermined organism comprising administering to that animal a vaccine containing immunogenic animal antigens capable of causing an immune response in that animal against the desired organism together with a vector containing a gene encoding an accessory molecule ligand. The present invention also contemplates methods of vaccinating an animal which include administering the genes which encode the immunogenic antigen capable of causing a desired immune response or altering the immune response to a particular antigen together with a vector containing a gene including the accessory molecule ligand gene. In this particular embodiment, the vector or vectors introduced encode the immunogenic antigens desired and the desired accessory molecule ligand. The present invention also contemplates that the gene or genes encoding the immunogenic peptide or peptides may be present on the same vector as is the gene or genes encoding the accessory molecule ligand.

The vaccination methods of the present invention are general in that they may be used to produce a vaccination against any predetermined organism, such as a virus, a bacteria, a fungus or other organism. In addition, the present vaccination methods may be used to produce an immune response against a neoplastic cell.

In other preferred embodiments, the vaccination methods of the present invention utilize a genetic vector, a genetic construct or a gene therapy vector which contains an accessory molecule ligand gene which is a chimeric accessory molecule ligand gene. That chimeric accessory molecule ligand gene preferably contains at least a portion of the murine CD40 ligand gene. In other preferred embodiments, the vaccination method utilizes a DNA molecule which encodes at the

minimum the accessory molecule ligand gene or a chimeric accessory molecule ligand gene. This particular DNA may or may not include a promoter sequence which directs the expression of the accessory molecule ligand gene.

5       The present invention also contemplates that the vaccination method may utilize a genetic vector which is capable of expressing an accessory molecule ligand within a particular cell or organism together with a vector which is capable of expressing at least a single  
10 polypeptide from an andovirus. This andovirus polypeptide may be expressed from the same or different vector which expresses the accessory molecule ligand in that cell. In this particular embodiment, the andovirus polypeptide is also expressed in at least one cell type  
15 within the organism and serves to modulate the immune response found in response to this vaccination protocol.

      The present invention also contemplates the introduction of an accessory molecule ligand gene into cells which are present in the joints of patients with  
20 rheumatoid arthritis. In preferred embodiments, the accessory molecule ligand gene introduced comprises at least a portion of the Fas ligand gene and upon expression the accessory ligand induces the cell death of cells expressing Fas on the cell surface. This  
25 process leads to the reduction of the destructive inflammatory process.

      The following examples are provided to illustrate various aspects of the present invention and do not limit the scope of that invention.

30

#### VII. Methods of Treating Arthritis

      The present invention also contemplates methods of treating arthritis comprising inserting into a joint,  
35 cells which have been transformed with an accessory

1 molecule, such as the Fas ligand. In preferred  
embodiments, the expression of that accessory molecule  
ligand or the stability of that molecule on the surface  
of the cells has been altered. In these preferred  
5 embodiments, the accessory molecule ligand functions in  
an enhanced manner to aid in the treatment of arthritis  
within the joint. The present invention contemplates  
treating human arthritis both in vivo, ex vivo, and by  
directly injecting various DNA molecules containing  
10 genes which encode the useful accessory molecule ligand  
into the patients. Various useful protocols may be  
designed to rheumatoid arthritis including those  
described in the example section below.

The present invention contemplates the treatment of  
15 arthritis utilizing accessory molecule ligand genes  
which may be chimeric accessory molecule ligand genes  
comprised of portions of that gene being derived from  
two different accessory molecule ligand genes. In other  
embodiments, the chimeric accessory molecule ligands may  
20 be produced by utilizing domains from the same accessory  
molecule ligand gene. The resulting chimeric accessory  
molecule ligands have an altered stability on the  
surface of cells upon which they are expressed. This  
altered stability modulates the function of the immune  
25 system in the local environment around the cells in  
which these chimeric accessory molecule ligands are  
expressed. For example, in certain preferred  
embodiments, Fas ligand stability is altered on the  
surface of cells within a joint of a patient suffering  
30 from arthritis. This altered stability modulates the  
immune system and causes the cells to be targeted for  
apoptosis and thus reducing the immune response within  
the inflamed joint. In other embodiments, the accessory  
molecule ligand genes described within are altered such  
35 that the resulting accessory molecule ligand has an

altered stability and causes an immunomodulatory effect which can be useful in the treatment of arthritis.

The present invention contemplates in preferred embodiments that chimeric accessory molecule ligands  
5 genes be utilized in the treatment of arthritis. These chimeric accessory molecule ligand genes preferably contain at least a portion of the Fas ligand gene Domain IV, which carries the effect or function for Fas ligand. In preferred embodiments, at least in the portion of  
10 that domain, is present which allows Fas ligand to have its biologic effects. In other preferred chimeric accessory molecule ligands, those ligands contain domains from other accessory molecule ligand genes of the present invention or from a different domain of the  
15 same accessory molecule ligand. Particularly preferred are Fas chimeric accessory molecule ligand genes made up on Domain IV of the human Fas ligand operatively linked with Domain III of the mouse Fas ligand. This particular combination results in more stable Fas ligand  
20 and thus, by replacing Domain III of human Fas ligand with Domain III of the mouse ligand, the activity of the human Fas ligand gene is altered.

Alternatively, in other preferred embodiments, the murine Fas ligand gene is used to encode the murine Fas  
25 ligand on the surface of cells in place of the human Fas ligand. The murine Fas ligand is more stable than the human Fas ligand and thus, alters the Fas ligand activity in the joint. The resulting alter Fas ligand activity is useful in the treatment of rheumatoid  
30 arthritis.

Further preferred embodiments include embodiments in which the effect or function present on Domain IV of the human Fas ligand is combined with other domains from other accessory molecule ligands. For example,  
35 CD70 Domain III is more stable than Domain III of the

human Fas ligand and thus the chimeric accessory molecule ligand made up of Domain III from the human CD70 and Domain IV of the Fas ligand together with other supporting domains would be more stable. The increased stability leads to increase Fas ligand activity. In other preferred embodiments, Domain III of the Fas ligand is replaced with multiple copies of a domain or domains. Such multiple copies of domains include domains made up of two or more copies of other domains such as Domains III or I of the CD70 molecule.

In other preferred embodiments, the present invention contemplates accessory molecule ligand genes, such as Fas ligand genes, in which a cleavage site for matrix-metalloproteinase (MMP), have been removed from the accessory molecule ligand. MMP cleavage and recognition sites, charted in Figure 28, are discussed in Smith, M.M. et al., Journal of Biol. Chem. 270:6440-6449 (95) and Nagase, H., and G.B. Fields, Biopolymers (Peptide Science) 40:399-416 (96). In preferred embodiments, at least one MMP site has been removed from at least Domain III of the Fas ligand gene. The removal of the MMP site from the Fas ligand gene makes the Fas ligand more stable and thus, more effective in the treatment of arthritis.

In other preferred embodiments, chimeric accessory molecule ligand genes are comprised of portions of the human Fas ligand gene with other domains from other human accessory molecule ligands or domains from accessory molecules derived from other species. For example, the present invention contemplates the use of domains from CD40 ligand, CD70 ligand, CD30 ligand, TNF-related apoptosis inducing ligand (TRAIL), TNF- $\alpha$  as well as mutants of human Fas ligand and murine Fas ligand. Production of such chimeric accessory molecule ligands is easily accomplished by manipulating and producing

accessory molecule ligand genes which are chimeric and thus has portions derived from at least two different accessory molecule ligand genes.

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EXAMPLES1. Expression of Human and Mouse Accessory  
Molecule Ligand in Human CLL Cellsa. Construction of a Genetic Construct and Gene  
Therapy Vector Containing a Human and Mouse  
Accessory Molecule Ligand Gene

Either the human accessory molecule ligand gene (human CD40 ligand) or the murine accessory molecule ligand gene (murine CD40 ligand) was constructed utilizing the respective human and murine genes. Each of these genes was cloned in the following manner.

i. Murine CD40-L cloning

Total RNA was isolated using the RNA STAT-60 kit (Tel-Test "B" Inc., Friendswood, TX) from 1 x 10<sup>7</sup> B6 mouse splenocytes that were previously activated for 8 hours with immobilized CD3-specific mAb. cDNA was then synthesized with the Superscript cDNA synthesis kit (Gibco BRL, Grand Island, NY) using oligo-dT primers. The murine CD40 ligand (mCD40-L) gene was then amplified from the cDNA by PCR using the following mCD40-L specific primers. 5'-GTTAAGCTTTTCAGTCAGCATGATAGAA (SEQ ID NO: 26), 5'-GTTTCTAGATCAGAGTTTGAGTAAGCC (SEQ ID NO: 27). The amplified mCD40-L PCR product was subcloned into the HindIII and XbaI sites of the eukaryotic expression vector pcDNA3 (Invitrogen, San Diego, CA). A DNA fragment encompassing the CMV promoter, mCD40-L gene, and polyadenylation signal was released from this plasmid construct after restriction digestion with BglII and XhoI enzymes. This DNA fragment was then subcloned into the shuttle plasmid MCS(SK)pXCX2 (Spessot R, 1989,



Virology 168:378) that was designated mCD40-L pXCX2. This plasmid was used for adenovirus production as described below.

5           ii. Human CD40-L Cloning

A plasmid containing the gene for human CD40-L was used to produce the human CD40-L gene used herein. The sequence of this gene is available and thus this source  
10 of the gene was used merely for convenience. See GenBank accession no. X67878. This plasmid was used for PCR amplification of the human CD40-L gene using the specific primers, sense primer 5' CCAAGACTAGTTAACACAGCATGATCGAAA 3' (SEQ ID NO: 28) and  
15 antisense primer 5' CCAATGCGGCCGCACTCAGAATTCAACCTG 3' (SEQ ID NO: 29).

These primers contain flanking restriction enzyme sites for subcloning into the eukaryotic expression plasmid pRc/CMV (Invitrogen). The PCR amplified CD40-L  
20 fragment was subcloned into the SpeI and NotI sites of pRc/CMV and designated hCD40-L pRc/CMV. A BglII and XhoI fragment encompassing the CMV promoter, hCD40-L gene, and polyadenylation signal was then released from this plasmid and subcloned into the shuttle plasmid  
25 MCS(SK)pXCX2 as described above. This plasmid was designated hCD40-L pXCX2. This plasmid was used for adenovirus production as described below.

30           iii. Adenovirus Synthesis

Either mCD40-L pXCX2 or hCD40-L pXCX2 plasmids were co-transfected with pJM17 (Graham and Prevec, 1991, Methods in Molecular Biology, Vol 7) into 293 cells (American Type Culture Collection, Rockville, MD) using  
35 the calcium phosphate method (Sambrook, Fritsch, and

Maniatis, 1989, Molecular Cloning, A Laboratory Manual,  
2nd edition, chapter 16:33-34). Isolated adenovirus  
plaques were picked and expanded by again infecting 293  
cells. High titer adenovirus preparations were obtained  
5 as described (Graham and Prevec, 1991, Methods in  
Molecular Biology, Vol 7), except for the following  
modifications. The cesium chloride gradient used for  
concentrating viral particles was a step gradient, with  
densities of 1.45 g/cm<sup>3</sup> and 1.2 g/cm<sup>3</sup>. The samples were  
10 spun in a SW41 rotor (Beckman, Brea, CA) at 25,000 rpm  
at 4°C. The viral band was desalted using a Sephadex  
G25 DNA grade column (Pharmacia, Piscataway, NJ). The  
isolated virus was stored at 70°C in phosphate buffered  
saline with 10% glycerol. The virus titer was  
15 determined by infecting 293 cells with serial dilutions  
of the purified adenovirus and counting the number of  
plaques formed. Viral titers typically ranged from 10<sup>10</sup>  
to 10<sup>12</sup> plaque forming units/ml (PFU/ml).

20        b.    Introduction of a Murine and Human Accessory  
Molecule Ligand Gene into CLL Cells and HeLa  
Cells

For adenovirus infection, 10<sup>6</sup> freshly thawed and  
25 washed CLL cells or HeLa cells were suspended in 0.5 to  
1 mL of culture medium for culture at 37°C in a 5% CO<sub>2</sub>-  
in-air incubator. Adenovirus was added to the cells at  
varying multiplicity of infection (MOI), and the  
infected cells were cultured for 48 hours, unless  
30 otherwise stated, before being analyzed for transgene  
expression.

c. Expression of an Accessory Molecule Ligand  
Gene in CLL Cells and HeLa Cells

5 The CLL and HeLa cells which were infected with the  
adenovirus vector containing either mouse or human CD40  
ligand genes prepared in Example 1b. were then stained  
with commercially available monoclonal antibodies  
immunospecific for either human or mouse CD40 ligand  
(Pharmingen, San Diego, CA) using the manufacturer's  
10 directions. The CLL and HeLa cells were washed in  
staining media (SM) consisting of RPMI-1640, 3% fetal  
calf serum and 0.05% sodium azide and containing  
propidium iodide and then analyzed on a FACScan (Becton  
Dickinson, San Jose, CA). Dead cells and debris were  
15 excluded from analysis by characteristic forward and  
side light scatter profiles and propidium iodide  
staining. Surface antigen expression was measured as  
the mean fluorescence intensity ratio (MFIR). MFIR  
equals the mean fluorescence intensity (MFI) of cells  
20 stained with a specific FITC-conjugated MoAb, divided by  
the MFI of cells stained with a control IgG-FITC. This  
method controls for the nonspecific increases in auto-  
fluorescence seen in larger, more activated cells.

The histograms, generated for the CLL cells and  
25 HeLa cells containing either a genetic vector containing  
the human CD40 ligand gene or the murine CD40 ligand  
gene and the appropriate controls, are shown in Figure  
3A-3D. The expression of both the murine and human  
accessory molecule ligand gene (CD40 ligand) in HeLa  
30 cells is shown in Figures 3A and 3B, respectively. The  
expression of the murine and human accessory molecule  
ligand in CLL cells is shown in Figures 3C and 3D. The  
expression of an accessory molecule ligand gene in CLL  
cells and the expression of murine CD40 ligand on the  
35 surface of the CLL cells is shown in Figure 3C. The

failure of the human accessory molecule ligand to be expressed on the surface of the CLL cells is shown in Figure 3D.

Figure 8 shows data from an experiment done to  
5 examine whether the CD4<sup>+</sup> T cells of CLL patients could be induced to express the accessory molecule ligand mRNA after CD3 ligation. An ELISA-based quantitative competitive RT-PCR was used to measure CD40 ligand transcript levels. In this experiment, CD40 ligand and  
10 RNA transcribed from the CD40 ligand gene in CLL cells are compared with levels of CD40 ligand and RNA made in normal donor cells, after induction by CD3 ligation. For CD3 activation, plate coats of CD3 mAb were made and incubated with plated CLL or normal donor mononuclear  
15 cells for the indicated amount of time, after which cells were analyzed for expression of surface antigens or CD154 RNA message levels. CLL or normal donor serum was added to the cells at the beginning of the activation assay for examination of modulation of CD40  
20 ligand surface expression.

For quantitative CD154 RT-PCR ELISA, total RNA was extracted and competitor RNA was generated from the insert containing CD40 ligand (CD154) cDNA. Varying  
25 amounts of competitor RNA were added to separate wells of isolated total RNA that subsequently were converted into cDNA. CD3 activation, ELISAs and PCR reactions were performed as described in Cantwell, M. et al., Nature Medicine 3:984-989 (1997). Biotinylated PCR products were captured onto microtiter plates (Becton  
30 Dickinson, Oxnard, CA) coated with streptavidin (Sigma), and incubated. The plate was treated with NaOH to remove the sense strands and subsequently washed. The DNA was then hybridized with either wild-type gene-specific or competitor-specific oligonucleotides. Using  
35 terminal transferase, each probe was labeled with a

5 molecule of digoxigenin-11-dideoxyUTP (Boehringer  
Mannheim). The plate was incubated and washed with HYBE  
buffer and blocking buffer, then peroxidase-conjugated  
anti-digoxigenin antibody (150 U/ml; Boehringer  
10 Mannheim) in blocking buffer was added. TMB  
(tetramethylbenzidine) and peroxidase (Kirkegaard and  
Perry Laboratories, Gaithersburg, MD) were added for  
color development, and optical densities were measured  
at 450 nm and Deltasoft II (Biometallics, Princeton, NJ)  
15 was used for data analysis.

Standard curves plotting the moles of RNA product  
versus the optical density were made for the standard  
cDNA reactions. The equations describing these standard  
curves were then used to calculate the moles of wild-  
15 type or competitor DNA present in the unknown PCR  
reactions based on the optical densities obtained in the  
ELISA readings. The ratio of the quantity of wild-type  
DNA to the amount of competitor DNA was then plotted  
against the known quantity of competitor RNA added in  
20 the initial samples. The ratio of 1 was taken for the  
extrapolation of the amount of unknown moles of target  
RNA in the sample (a ratio of 1 means the amount of  
target RNA versus competitor RNA are equal). The  
molecules of target RNA per CD4 cell was then calculated  
25 based on the following formula: [(moles target CD154  
RNA) x (6 X 10<sup>23</sup> molecules/mole) x (dilution factor of  
test RNA)]/(% of CD4 T cells in total cell population).

The upper graph in Figure 8 shows that T cells of  
patients with CLL do not express detectable CD40 ligand  
30 after CD3 ligation. CD40 ligand RNA is produced, but it  
is not stable. Although both CD40 ligand and CD40  
ligand RNA are expressed in normal donor T cells (lower  
graph), the levels of neither the protein or RNA are  
stably maintained.

Figure 9 shows a time course for surface expression of CD40 ligand. Expression reached a peak level at 48 hours after infection and persisted at high levels for at least 6 days thereafter. In this experiment, CLL B cells were infected with a gene therapy vector containing an accessory molecule ligand, at a MOI of 1000 at time zero, and then assessed by flow cytometry at various times thereafter. At each time point listed on the abscissa, the proportions of viable CLL B cells that expressed detectable CD154 are indicated by the vertical bars corresponding to the percentage scale depicted on the right-hand ordinate.

d. Function of the Human and Murine Accessory Molecule Ligands

i. Induction of CD80 and CD54 on Cells Containing a Gene Therapy Vector Encoding an Accessory Molecule

The CLL cells infected with the murine accessory molecule ligand gene prepared in Example 1b. were then cultured in tissue culture plates. The CLL cells were then analyzed using multiparameter FACS analysis to detect induction of CD80 and CD54 expression using fluorescein isothiocyanate-conjugated monoclonal antibodies immunospecific for each of these respective surface antigens. Non-infected CLL cells were used as a control. The cells were subjected to the appropriate FACS analysis and histograms were generated. CD80 mAb was obtained from Dr. Edward Clark and CD54 mAb was purchased from CALTAG Inc. The CD80 was conjugated using standard methods which have been described in Kipps et al., Laboratory Immunology II, 12:237-275 (1992).

The results of this analysis are shown in Figure 4A-4D. Figures 4A-4B compare the amount of CD54 expression in CLL cells which have not been transfected (Figure 4A) or CLL cells into which a gene therapy vector containing the murine CD40 ligand gene was introduced (Figure 4B). The shaded graph indicates the isotype control for FACS staining and the open graph indicates the cells stained with the anti-CD54 antibody. These results show that the level of expression of CD54 is increased in CLL cells into which the gene therapy vector containing the murine CD40 ligand was introduced.

Figures 4C and 4D compare the amount of CD80 expression in CLL cells which have not been transfected (Figure 4C) or CLL cells into which a gene therapy vector containing the murine CD40 ligand gene was introduced (Figure 4D). The shaded graph indicates the isotype control for FACS staining and the open graph indicates the cells stained with the anti-CD80 antibody. These results show that the level of expression of CD80 is increased in the CLL cells into which the gene therapy vector containing the murine CD40 ligand was introduced.

In an additional experiment, CLL cells infected with a gene therapy vector containing the murine accessory molecule ligand gene were evaluated by flow cytometry for induced expression of not only CD54 and CD80, but also CD86, CD58, CD70 and CD95. Fluorescein-conjugated mAb specific for human CD54 and CD70 were purchased from CALTAG. Fluorescein-conjugated mAb specific for human CD27, CD58, CD80, CD86, or CD95, and phycoerythrin-conjugated mAb specific for human or mouse CD40 ligand, were obtained from PharMingen. Shaded histograms represent staining of CLL B cells with FITC-conjugated isotype nonspecific mAb. In contrast to uninfected CLL cells (Figure 10, thin-lined histograms),

or Ad-lacZ-infected CLL cells (data similar to that obtained with uninfected cells, but not shown), CLL cells infected with the adenovirus vector encoding the CD40 ligand (CD154) expressed high levels of CD54 (Figure 10, top left), CD80 (Figure 10, top middle), CD86 (Figure 10, top right), CD58 (Figure 10, bottom left), CD70 (Figure 10, bottom middle), and CD95 (Figure 10, bottom right). On the other hand, CD40 ligand-CLL (CD154 CLL) expressed significantly lower levels of both surface membrane CD27 (Figure 11A, thick-lined histogram) and soluble CD27 (Figure 11B) than uninfected (Figure 11A, thin-lined histogram) ( $P < 0.01$ , Bonferroni t-test) or Ad-lacZ-infected CLL cells (data similar to that obtained with uninfected cells, but not shown). In the experiment shown in Figure 11A, the CLL B cells were examined for expression of CD27 via flow cytometry, three days after infection. Shaded histograms represent staining of CLL B cells with FITC-conjugated isotype control mAb. In Figure 11B, cell-free supernatants were collected, after the infection or stimulation of CLL B cells, for 72 hours and tested for the concentration of human CD27 by ELISA. The reduced expression of CD27 (Figure 11B) is similar to that noted for leukemia B cells stimulated via CD40 cross-linking with mAb G28-5 presented by CD32-expressing L cells, as described in Rassenti, L.Z. and T.J. Kipps, J. Exp. Med. 185:1435-1445.

ii. Allogeneic T Cell Responses to CLL Cells Into Which a Genetic Therapy Vector Containing a Murine CD40 Ligand Gene Has Been Introduced

The ability of CLL cells which have been infected with a gene therapy vector containing the murine CD40



ligand gene to stimulate allogeneic T cells (i.e., from another individual) was analyzed using cell proliferation assays. Briefly, the test cells were co-cultured with the genetic therapy vector containing the lac-Z gene or the murine CD40 ligand gene at a multiplicity of infection of 1,000 in the presence of IL-4 at a concentration of 10 ng/ml. In other samples, the CLL cells were stimulated with MOPC21 (a control IgG) or G28-5 (an anti-CD40 monoclonal antibody) or were preincubated on CD32-L cells and at the same time treated with IL-4. The preincubation with the CD32-L cells together with IL-4 treatment have been shown to be an efficient form of cross-linking the CD40 molecule other than direct gene transfection.

After three days of culture at 37°C, these cells were treated with mitomycin C to prevent their proliferation and then used to stimulate allogeneic T cells. Prior to this co-culture, the different aliquots of CLL cells had either been treated with the anti-CD40 monoclonal antibody or had been infected with the gene therapy vector containing either the lac-Z or murine CD40 ligand gene at a stimulator ratio of 1:10. After two days of culture at 37°C, interferon gamma (IFN $\gamma$ ) production was measured by ELISA assay. After five days of co-culture at 37°C, the incorporation of <sup>3</sup>H-thymidine into replicating cells was measured after an eight hour pulse label. The results of this assay are shown in Table II below and in Figure 5.

In another experiment, CLL B cells infected with the gene therapy vector containing the CD40 ligand gene were evaluated for their ability to act as stimulator cells in an allogeneic mixed lymphocyte T cell reaction (MLTR). In parallel, the stimulatory capacity of control lac-Z-vector-infected CLL cells and CLL B cells that had been cultured with CD32-L cells and an anti-

CD40 mAb (G28-5) or an isotype control Ig, was also examined as described in Ranheim, E.A. and T.J. Kipps, J. Exp. Med., 177:925-935 (1993), Clark, E.A. and J.A. Ledbetter, Proc. Natl. Acad. Sci. USA, 83:4494-4498 (1986), and Banchereau, J. et al., Science 251:70-72 (1991). Effector T cells from a non-related donor were co-cultured with the CLL stimulator cells at an effector to target ratio of 4:1. After 18 h culture at 37°C, over 30% of the allogeneic CD3<sup>+</sup> cells were found to express the activation-associated antigen CD69 when cultured with CD154-CLL cells (data not shown). In contrast, less than 4% of the T cells expressed CD69 when co-cultured with uninfected or Ad-lacZ-infected CLL cells (data not shown).

Two days after the initiation of the MLTR, the concentrations of IFN $\gamma$  in the culture supernatants were assayed by ELISA. The supernatants of the MLTR stimulated with CLL cells infected with the accessory molecule ligand CD40L (Figure 12A, CD154-CLL) contained significantly higher levels of IFN $\gamma$  ( $306 \pm 5$  ng/ml,  $m \pm SE$ ,  $n = 3$ ) than that of MLTR cultures stimulated with the anti-CD40 mAb (Figure 12A,  $\alpha$ CD40-CLL) ( $23 \pm 3$  ng/ml) ( $P < 0.05$ , Bonferroni t-test). The latter was not significantly different from that of MLTR cultures stimulated with control Ad-lacZ-infected CLL cells (Figure 12A, lacZ-CLL) ( $43 \pm 10$  ng/ml) ( $P > 0.1$ , Bonferroni t-test). The supernatants of effector cells alone, or of MLTR cultures stimulated with uninfected CLL cells (Figure 12A, CLL) or control Ig treated CLL cells (Figure 12A, MOPC-CLL), did not contain detectable amounts of IFN $\gamma$  ( $<2$  ng/ml). Similarly, none of the leukemia B cell populations produced detectable amounts of IFN $\gamma$  when cultured alone, without added effector T cells (data not shown).

After 5 days, cell proliferation was assessed by incorporation of  $^3\text{H}$ -thymidine. Cultures with isotype control IgG-treated (Figure 12B, MOPC-CLL) or uninfected (Figure 12B, CLL) stimulator cells did not incorporate more  $^3\text{H}$ -thymidine than cultures without added leukemia-stimulator cells (Figure 12B, None). Ad-lacZ-infected CLL B cells (Figure 12B, lacZ-CLL) also were unable to stimulate allogeneic T cells to incorporate amounts of  $^3\text{H}$ -thymidine that were much greater than that of control cultures. In contrast, anti-CD40-stimulated leukemia cells or CD154-CLL cells each induced significant effector cell proliferation (Figure 12B,  $\alpha\text{CD40}$ -CLL or CD154-CLL) ( $P < 0.05$ , Bonferroni t-test). Moreover, the amount of  $^3\text{H}$ -thymidine incorporated by cultures stimulated with CD154-CLL cells ( $41,004 \pm 761$  cpm ( $m \pm \text{SE}$ ),  $n = 3$ ) was significantly greater than that of cultures stimulated with equal numbers of  $\alpha\text{CD40}$ -CLL cells ( $22,935 \pm 1,892$  cpm;  $n = 3$ ) ( $P < 0.05$ , Bonferroni t test). However, neither of these mitomycin-C-treated leukemia cell populations incorporated  $^3\text{H}$ -thymidine when cultured without effector T cells (data not shown). Also, as described for the MLTR between allogeneic T cells and CD40-stimulated CLL cells {6549, 7167, 7168}, allogeneic T cell proliferation in response to CD154-CLL could be inhibited by CTLA-4-Ig or CD11a mAb when added at the initiation of the MLTR, indicating that respective interactions between CD80/CD86 and CD28, or CD54 and CD11a/CD18, contribute to the noted allogeneic T cell reaction (data not shown).

30

Table IIAllogeneic T cell responses to CLL cells  
infected with mCD40-L adenovirus

5

	Stimulators	<u>% positive cells</u>		<u>Allogeneic response</u> <u>(mean±SEM)</u>	
		mCD40-L	Human CD80	3H-TdR uptake (cpm)	IFN $\gamma$ production (ng/ml)
10	None (t cells only)	-	-	3577 ± 821	n.d.*
	CLL with:				
	No activation	0	1.4	4577 ± 1097	n.d.
	MOPC21	0	1.0	5259 ± 1788	n.d.
	G28-5	0	26.7	22935 ± 1892	22.3 ± 1.6
15	lac-Z adeno	0	4.8	9037 ± 1781	43.2 ± 10.5
	mCD40-L adeno	17.5	19.7	41004 ± 761	305.7 ± 4.5
* n.d. - not detectable					

iii. Stimulation of Gamma Interferon by CLL  
Cells Containing an Accessory Molecule  
Ligand Gene

20

The function of CLL cells containing an accessory molecule ligand gene (mouse CD40 ligand) was analyzed by determining the ability of those cells to activate T lymphocytes. The procedure was performed as follows: allogeneic T lymphocytes from a healthy donor (greater than 90% CD3<sup>+</sup>) were purified using magnetic beads and monoclonal antibodies specific for the CD14 and CD19 antigen. These allogeneic T lymphocytes then were cultured together with MMC-treated CLL cells which were infected with the accessory molecule ligand gene (murine CD40 ligand) or the lac-Z gene. This co-culture was performed in RPMI-1640 medium containing 10% fetal calf serum. After culture for 24 hours, the cells were

15 As can be seen, the human CLL cells containing the accessory molecule ligand gene (murine CD40) produced substantially higher concentrations of interferon gamma in the cell culture supernatant when compared to CLL cells which contained the lac-Z gene. The increased  
20 production of interferon gamma (IFN $\gamma$ ) by T lymphocytes exposed to CLL cells containing the accessory molecule ligand gene indicates that these CLL cells containing the accessory molecule ligand genes were effective in producing an enhanced immune response.

iv. Stimulation of Allogeneic T Cells Pre-Exposed to Non-Modified CLL B Cells Containing an Accessory Molecule Ligand Gene

30 Prior studies indicated that antigen presentation to T cells, in the absence of the signals derived from costimulatory molecules such as CD28, can lead to specific T cell clonal anergy. For this reason,  
35 allogeneic T cells that had previously been cultured,

with non-modified CLL B cells lacking expression of CD80 and other immune accessory molecules, were tested for their ability to respond to CLL cells containing the CD40 ligand gene. Allogeneic effector cells did not  
5 incorporate more  $^3\text{H}$ -thymidine in response to non-modified CLL cells (Figure 12C, CLL), or control CLL cells infected with Ad-lacZ (Figure 12C, lacZ-CLL), than when they were cultured alone (Figure 12C, None). In contrast, even after prior co-culture with non-modified  
10 CLL B cells, allogeneic effector cells could still be induced to proliferate (Figure 12C, CD154-CLL) or to produce  $\text{IFN}\gamma$  (Figure 12D, CD154 CLL) in response to cells expressing an accessory molecule ligand. Although modest amounts of  $\text{IFN}\gamma$  were detected in the supernatants  
15 of such secondary cultures when Ad-lacZ-infected leukemia cells were used as stimulator cells (Figure 12D, lacZ-CLL), this level was significantly lower than that noted for secondary cultures with Ad-CD40-ligand-infected CLL cells (Figure 12D, CD154-CLL) ( $P < 0.05$ ,  
20 Bonferroni t-test). Similarly, the supernatants of the leukemia cells alone (data not shown), and the effector cells alone (Figure 12D, None), of the MLTR cultures stimulated with uninfected CLL cells (Figure 12D, CLL), contained negligible amounts of  $\text{IFN}\gamma$  ( $< 2 \text{ ng/ml}$ ). These  
25 results indicate that allogeneic effector cells cultured with nonmodified CLL B cells are not precluded from responding to CLL B cells infected with a gene therapy vector containing the accessory molecule ligand gene.

v. Autologous T Cell Responses to CLL Cells  
Into Which a Gene Therapy Vector Encoding  
a Murine Accessory Molecule Ligand Gene  
Has Been Introduced

5

6082222 12019  
T cells isolated from the blood of CLL patients were examined for their ability to respond in vitro to autologous CLL B cells containing a gene therapy vector which encodes the murine accessory molecule, CD40 ligand. T cells were isolated to >95% purity, and then co-cultured with mitomycin-C-treated autologous leukemia cells in serum-free AIM-V medium supplemented with exogenous interleukin-2 at 25 U/ml. Modest <sup>3</sup>H-thymidine incorporation ( $\leq 10,000$  cpm) was detected in cultures without added stimulator cells, secondary in part to the exogenous IL-2 (Figure 13A, and data not shown). The level of T cell proliferation, however, did not increase in response to uninfected CLL cells (Figure 13A, CLL) or Ad-lacZ-infected CLL cells (Figure 13A, lacZ-CLL). In contrast, CLL cells infected with a gene therapy vector containing the accessory molecule ligand (Figure 13A, CD154-CLL) induced autologous T cells to incorporate significantly more <sup>3</sup>H-thymidine ( $17,368 \pm 1,093$  cpm,  $n=3$ ) than any of the control cultures ( $P < 0.05$ , Bonferroni t-test). Furthermore, the MLTR stimulated with CLL cells infected with a vector encoding an accessory molecule ligand (CD40L) also generated significantly more IFN $\gamma$  ( $165 \pm 3$  ng/ml,  $n=3$ ) than any of the other cultures (Figure 13B) ( $P < 0.05$ , Bonferroni t-test).

The T cells were harvested after 5 days from the autologous MLTR and assessed for CTL activity against autologous CLL B cells. T cells co-cultured with autologous CD40-ligand-CLL cells developed CTL activity for non-modified CLL B cells, effecting 40.1% lysis ( $\pm$

2.3%) at an E:T ratio of 2:1 (Figure 13C, CD154). However, such T cells did not develop detectable CTL activity for the same target cells in the control reactions, when co-cultured with uninfected or Ad-lacZ-  
5 infected CLL cells (Figure 13C).

vi. Specificity of CTL Stimulated by  
Autologous CD40-Ligand-CLL B Cells for  
Allogeneic CLL B Cells

10 Effector cells stimulated with autologous CD40-ligand-CLL were evaluated for their ability to secrete IFN $\gamma$  or manifest CTL activity against allogeneic CLL B cells (Figure 14). After 5 days of autologous MLTR with  
15 CD154-CLL or lacZ-CLL, T cells were isolated by Ficoll density gradient centrifugation, washed extensively, and then cultured in media for 24 h. Washed T cells were mixed with autologous ("Auto CLL", solid bar) or  
allogeneic ("Allo-1 CLL" or "Allo-2 CLL", shaded or  
20 hatched bars) target CLL B cells. T cells stimulated in the autologous MLTR with CD40-ligand-CLL cells, but not with lacZ-CLL cells, produced significantly more IFN $\gamma$  in response to secondary culture with non-modified  
autologous CLL B cells than with allogeneic CLL B cells  
25 (Figure 14A) ( $P < 0.05$ , Bonferroni t-test). Furthermore, T cells stimulated with CD40-ligand-CLL cells, but not with lacZ-CLL cells, were cytotoxic for autologous CLL cells, but not allogeneic CLL cells (Figure 14B).  
Similar results were obtained with the autologous MLTR-  
30 activated T cells of the allogeneic donor, again demonstrating specific cytotoxicity for autologous CLL B cells (data not shown). Finally, W6/32, a mAb to class I major histocompatibility complex (MHC I) antigens  
could significantly inhibit the cytotoxicity of T cells  
35 stimulated with CD40-ligand-CLL cells for autologous CLL



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B cells (Figure 14C,  $\alpha$ HLA-class I) ) ( $P < 0.05$ , Bonferroni t-test). Such inhibition was not observed with mAb specific for MHC class II antigen (Figure 14C,  $\alpha$ HLA-DP), mAb specific for the Fas-ligand (Figure 14C,  $\alpha$ FasL), or an isotype control mAb of irrelevant specificity (Figure 14C, MOPC-21). Collectively, these studies indicate that Ad-CD40-ligand-infected CLL cells can induce an autologous anti-leukemia cellular immune response in vitro, leading to the generation of MHC-class I-restricted CTL specific for autologous non-modified leukemia B cells.

e. Transactivation of Non-Infected Bystander Leukemia B Cells by Ad-CD40L CLL Cells

To address whether the changes in tumor marker expression (described in section 1di.) resulted from intracellular versus intercellular stimulation, the effect of culture density on the induced expression of CD54 and CD80 following infection with adenovirus gene therapy vector encoding the accessory molecule ligand (CD40L, or CD154) was examined. After infection, CLL cells were cultured at standard high density (e.g.  $1 \times 10^6$  cells/ml) or low density (e.g.  $2 \times 10^5$  cells/ml) for 3 days at  $37^\circ \text{C}$ . Cells plated at high density contained homotypic aggregates, whereas cells plated at low density remained evenly dispersed and without substantial cell-cell contact (data not shown). Despite expressing similar levels of heterologous CD154, CD154-CLL B cells cultured at high density were induced to express higher levels of CD54 and CD80 than CD154-CLL cells cultured at low density (Figure 15A). The stimulation achieved at high density could be inhibited by culturing the cells with a hamster anti-mouse CD154 mAb capable of blocking CD40 $\leftrightarrow$ CD154 interactions

(Figure 15B,  $\alpha$ CD154 Ab). Collectively, these studies indicate that CD154-CLL cells can activate each other in *trans* and that surface expression of CD154 is necessary for optimal leukemia cell stimulation.

5 In addition, Ad-CD154-infected, uninfected, Ad-lacZ-infected, or G28-5-stimulated CLL cells were labeled with a green-fluorescence dye to examine whether CD154-CLL could stimulate non-infected bystander leukemia cells. Dye-labeled cells were used as  
10 stimulator cells for equal numbers of non-labeled syngeneic CLL B cells. After 2 days' culture, stimulator cells cultured by themselves retained the green-fluorescence dye, allowing such cells to be distinguished from non-labeled CLL cells by flow  
15 cytometry. Bystander (green-fluorescence-negative) CD19<sup>+</sup> CLL B cells were induced to express CD54 (Figure 15C, right histogram) or CD86 (Figure 15D, right histogram) when co-cultured with Ad-CD154-infected leukemia B cells, but not with mock infected CLL cells (Figures 15C and 15D, left histograms), G28-5-stimulated CLL cells,  
20 or Ad-lacZ-infected CLL cells (data not shown). As expected, these bystander (green-fluorescence-negative) CLL cells also were negative for heterologous CD154.

25 f. Treatment of Leukemia with Gene Therapy  
Vectors Encoding an Accessory Molecule Ligand

Figure 24 shows an outline for a clinical trial for testing treatment of B cell CLL with adenovirus gene  
30 therapy vectors encoding modified CD40 ligand. Leukemia cells harvested by pheresis are infected with replication-defective vectors that encode the modified CD40 ligand. Following expression of this protein, the cells will be administered back to the patient for the  
35 purpose of stimulating a host anti-leukemia-cell immune

response. This strategy is far superior to one that uses gene therapy to affect expression of only one immune stimulatory molecule on the leukemia cell surface. Indded, this strategy results in the leukemia cells expressing an array of immune-stimulatory accessory molecules and cytokines, as well as a molecule that can affect the same changes in leukemia cells of the patient that were never harvested.

2. Expression of Chimeric Accessory Molecule Ligand Genes

The chimeric accessory molecule ligand genes described below are prepared using standard techniques as described herein.

a. Preparation of Chimeric Accessory Molecule Ligand Genes Utilizing Domains from Two Different Accessory Molecule Genes

3  
The human CD40 ligand gene was isolated from RNA prepared from T cells which had been activated by an anti-CD3 monoclonal antibody using 5' and 3' primers together with well known PCR methods. Chimeric accessory molecule genes of human CD40 ligand and murine CD40 ligand are constructed from the newly cloned human CD40 ligand gene and mouse CD40 ligand gene described herein as ~~SEQ ID NO. 2~~ <sup>SEQ ID NOS. 1 AND 2</sup>. The transmembrane and cytoplasmic domains of human CD40 ligand genes are exchanged with those of the murine CD40 ligand gene and designated H(Ex)-M(Tm-Cy) CD40 ligand. These chimeric accessory molecule ligand genes are produced using the gene conversion technique described as SOEN which has been previously described by Horton, Mol. Biotechnol.,

B 3:93 (1995). A diagram depicting the chimeric accessory molecule ligand genes which are produced is shown in Figure 4<sup>2</sup>. The nucleotide sequences of each of these respective chimeric accessory molecule ligand genes is designated SEQ ID NOS: 3-7 as indicated in the Table below.

Table III

10	<u>Chimeric Accessory Molecule Ligand Gene</u>	<u>SEQ ID NO:</u>
	HuIC/HuTM/MuEX CD40-Ligand	SEQ ID NO: 3
	HuIC/MuTM/HuEX CD40-Ligand	SEQ ID NO: 4
15	HuIC/MuTM/MuEX CD40-Ligand	SEQ ID NO: 5
	MuIC/HuTM/HuEX CD40-Ligand	SEQ ID NO: 6
	MuIC/MuTM/HuEX CD40-Ligand	SEQ ID NO: 7

Adenovirus vectors encoding each of the chimeric accessory molecules shown in Figure 2 are constructed using the methods described in Example 1. Each of these constructs are then transfected into either HeLa cells or CLL cells according to the methods of Example 1.

25      b.      Expression of Chimeric Accessory Molecule Ligands on CLL and HeLa Cells

The expression of each of the chimeric accessory molecule ligand genes constructed above is analyzed by using FACS analysis as specified in Example 1. The appropriate monoclonal antibody immunospecific for the external domain of either human or mouse CD40 ligand is selected and used to determine the level of expression of the chimeric accessory molecules on the surface of these cells. After appropriate analysis and preparation of appropriate histograms, the expression of chimeric accessory molecules containing at least a portion of the murine CD40 ligand gene is confirmed.

c. Function of Chimeric Accessory Molecule  
Ligands

CLL cells are infected with various MOI of the  
5 mCD40L adenovirus and then cultured in 48 or 24 well  
tissue culture plates for various times after infection  
(48, 72, and 96 hours). The CD19<sup>+</sup> B cells are then  
analyzed by multiparameter FACS analysis for induction  
of CD80 and CD54 expression using fluroescein  
10 isothiocyanate-conjugated mAb specific for each  
respective surface antigen as described in Example 1.  
Increased amounts of CD54 and CD80 are found on cells  
which have the chimeric accessory molecules containing  
the domain or domains derived from the mouse CD40 ligand  
15 gene.

Further analysis of the cells containing the  
chimeric accessory molecule genes is carried out  
according to Example 1(d). The cells containing the  
chimeric accessory molecule genes which contain the  
20 domains derived from the murine CD40 ligand gene are  
able to stimulate the production of gamma interferon and  
T cell proliferation.

25 d. Expression of Chimeric Accessory Molecule  
Genes Which Contain Proximal Extracellular  
Domains from Two Different Accessory Molecules  
from the Same Species

A chimeric accessory molecule ligand gene is  
30 prepared which contains the proximal extracellular  
domain from the human CD70 gene (Domain III) with the  
remainder of the domains derived from the human CD40  
ligand gene. This gene is prepared using standard  
biologic techniques as previously described herein.  
35 This chimeric accessory molecule ligand gene has the DNA

sequence shown as SEQ ID NO: 19. A different chimeric accessory molecule ligand gene is prepared which contains the proximal extracellular domain from the murine CD40 ligand gene with the remainder of the domains derived from the human CD40 ligand gene. This gene is prepared using standard techniques as previously described herein. This chimeric accessory molecule ligand gene has the DNA sequence shown as SEQ ID NO: 20.

The chimeric accessory molecule genes shown as SEQ ID NOS: 19 and 20 are inserted into the appropriate vectors as described in Example 1 and introduced into human neoplastic cells. The expression of that chimeric accessory molecule gene in the cells is determined as was described in Example 1.

The chimeric accessory molecule encoded by each of these chimeric accessory molecule genes is found on the surface of the human neoplastic cells using the FACS analysis described in Example 1. Increased amounts of CD54 and CD80 are found on the cells containing the chimeric accessory molecule genes using the techniques described in Example 1. The cells containing the chimeric accessory molecule gene are able to stimulate the production of gamma interferon and T cell proliferation as described and assayed according to Example 1.

### 3. Augmentation of Vaccination Using Vectors Encoding Accessory Molecules

The following procedures were used to demonstrate the augmentation of a vaccination protocol using a gene therapy vector encoding an accessory molecule.

a. Augmentation of the Antibody Response in  
Mice Co-Injected with an Accessory  
Molecule Gene Therapy Vector and placZ

5 Three different gene therapy constructs were prepared using standard techniques including those techniques described herein. The first was a control gene therapy vector, pcDNA3, which did not contain any gene. The second, placZ, contained the Lac-Z gene which  
10 encoded  $\beta$ -galactosidase ( $\beta$ -gal). The third, p-mCD40L, contained the murine CD40 ligand gene described in Example 1.

Prior to any immunizations, serum was isolated from 6-8 week old BALB/c-mice to determine the amount of any  
15 initial antibodies to  $\beta$ -galactosidase. Each animal was injected i.m. with 100 micrograms of plasmid DNA per injection. Four separate injections were given at one week intervals.

Prior to the third injection, the animals were bled  
20 to monitor the early antibody response to  $\beta$ -gal. One week after the final injection of plasmid DNA, the animals were bled to monitor the late antibody response to beta-galactosidase. To test the sensitivity of the assay, known amounts of anti- $\beta$ -gal antibodies isolated  
25 from an anti- $\beta$ -gal antiserum were tested in parallel.

Serum dilutions of 1:40, 1:200, or 1:1000 were tested in an ELISA for anti- $\beta$ -gal antibodies. For this, polystyrene microtiter ELISA plates were coated with  $\beta$ -gal at 10 microgram/ml in phosphate buffered saline.  
30 The plates were washed thrice with blocking buffer containing 1% bovine serum albumin (BSA), 0.2% Tween 20 in borate buffered saline (BBS) (0.1M borate, 0.2M NaCl, pH 8.2). 50 microliters of diluted serum were added to separate wells. After at least 1 hour at room  
35 temperature, the plates were washed thrice with blocking

10        The data for each of two experiments are provided  
in Tables IV and V which follow on separate sheets. The  
results are summarized in Tables VI and VII collating  
the data from the two experiments is provided as well.  
On the summary page n stands for the number of animals  
15 in each of the four groups. S.D. stands for standard  
deviation and Avg. is the average O.D. reading for all  
the animals in a particular group.

The results of Group 4 demonstrate that the use of a gene therapy vector encoding an accessory molecule ligand (CD40L) enhances the immunization against  $\beta$ -gal encoded by a genetic or gene therapy vector. The average O.D. reading of the 1:40 dilution of the sera from animals of this group is significantly higher than that of groups 1, 2, and 3 ( $P < 0.05$ , Bonferroni t tests, see Table VII).

SSSD/66239. v01



produce detectable antibodies to  $\beta$ -gal (Figure 16A). Mice were injected with either 100  $\mu$ g pcDNA3 (checkered bar), 50  $\mu$ g pcDNA3 + 50  $\mu$ g pCD40L (lined bar), 50  $\mu$ g pcDNA3 + 50  $\mu$ g placZ (striped bar), or 50  $\mu$ g pCD40L + 50  $\mu$ g placZ (solid bar). On the other hand, mice that received placZ and pcDNA3 developed detectable anti- $\beta$ -gal antibodies one week after the fourth and final injection, at d28. Mice that received placZ and pCD40L developed higher titers of anti- $\beta$ -gal antibodies than mice injected with placZ and pcDNA3. Figure 16B, ELISA analyses of serial dilutions of sera collected at d28, shows that mice co-injected with placZ and pCD40L had an eight-fold higher mean titer of anti- $\beta$ -gal antibodies at d28 than mice treated with placZ + pcDNA3.

i. Immunoglobulin Subclass Production  
Stimulated by Accessory Molecule Vector  
Co-Injection

Despite enhancing the titer of the anti- $\beta$ -gal antibody response, the subclass of anti- $\beta$ -gal IgG induced by injection of placZ was not altered by the co-injection of pCD40L. IgG<sub>2a</sub> anti- $\beta$ -gal antibodies predominated over IgG<sub>1</sub> subclass antibodies in the sera of mice injected with either placZ and pcDNA3 or placZ and pCD40L (Figure 17). Also depicted are the ELISA O.D. measurements of anti- $\beta$ -gal IgG<sub>1</sub> and anti- $\beta$ -gal IgG<sub>2a</sub> present in the pre-immune sera (striped bar) or post-immune sera (solid bar), collected at d28) of each group of mice, injected as indicated on the abscissa. In contrast, BALB/c mice injected with  $\beta$ -gal protein developed predominantly IgG<sub>1</sub> anti- $\beta$ -gal antibodies, and no detectable IgG<sub>2a</sub> anti- $\beta$ -gal antibodies.

ii. Augmentation of Vaccination by Accessory Molecule Vector Requires Co-Injection with placZ at the Same Site

5       The adjuvant effect of the pCD40L plasmid on the anti- $\beta$ -gal antibody response was noted only when it was injected into the same site as placZ (Figure 18). Groups of BALB/c mice (n=4) received intramuscular injections of placZ and pCD40L together at the same  
10 site, or as simultaneous separate injections at distal sites (right and left hind leg quadriceps). A control group received intramuscular injections of placZ and pcDNA3 at the same site. Animals were bled at d28 and the sera tested for anti- $\beta$ -gal Ab at different  
15 dilutions, as indicated on the abscissa. The graph illustrates a representative experiment depicting the mean O.D. at 405 nm of replicate wells of each of the serum samples for each group, at a 1:40, 1:200, or 1:1000 dilution. Animals injected simultaneously with  
20 placZ and pCD40L, but at different sites, did not develop detectable anti- $\beta$ -gal antibodies until d28. Moreover, the anti- $\beta$ -gal antibody titers of the sera from such animals at d28 were similar to that of mice that received placZ and pcDNA3, and significantly less  
25 than that of animals that received placZ and pCD40L together at the same site.

iii. Augmentation of Vaccination When Accessory Molecule Vector and placZ are Co-Injected into Dermis

30       The pCD40L plasmid also enhanced the anti- $\beta$ -gal antibody response to placZ when injected into the dermis. In the experiment shown in Figure 19, mice  
35 received intradermal injections, near the base of the

tail, with either 50  $\mu$ g pCDNA3 (checkered bar), 25  $\mu$ g pCDNA3 + 25  $\mu$ g pCD40L (lined bar), 25  $\mu$ g pCDNA3 + 25  $\mu$ g placZ (striped bar), or 25  $\mu$ g pCD40L + 25  $\mu$ g placZ (solid bar). Injections, bleeds and ELISA analyses were  
5 performed as in Figure 16A. The checkered bar and lined bar groups each consisted of 8 mice while the striped bar and solid bar groups each consisted of 12 mice. The height of each bar represents the mean O.D. of sera at a 1:40 dilution of each group  $\pm$  S.E. A statistical  
10 analysis of the data indicated that the striped bar and solid bar groups are independent ( $P < .05$ ). As observed with intramuscular injection, mice co-injected with placZ and pCD40L developed detectable serum anti- $\beta$ -gal antibodies one week following the second injection  
15 (d14), and two weeks earlier than mice injected with placZ and pCDNA3. Moreover, these animals also had an eight-fold higher mean titer of anti- $\beta$ -gal antibodies than mice of the placZ-injected group at d28. Mice injected with either the non-modified pCDNA3 vector or  
20 pCD40L alone did not produce detectable antibodies to  $\beta$ -gal.

25      b.    Augmentation of the CTL Response in Mice Co-Injected with an Accessory Molecule Gene Therapy Vector and placZ

The ability of pCD40L to enhance induction, by placZ, of CTL specific for syngeneic  $\beta$ -gal-expressing target cells was tested. BALB/c mice co-injected with  
30 pCD40L and placZ into skeletal muscle (Figure 20A) or dermis (Figure 20B) generated greater numbers of CTL specific for P13.2, a placZ transfected P815 cell line, than mice co-injected with placZ and pCDNA3. At a 5:1 effector:target ratio, the splenocyte effector cells  
35 from mice that received intramuscular injections of

placZ and pCD40L achieved greater than 20% specific lysis of P13.2. In contrast, when splenocytes of mice that received the control injection with placZ and pCDNA3 were used, a 9-fold greater ratio of effector to target cells was required to achieve this level of specific lysis. Similarly, the splenocyte effector cells from mice that received intradermal injections of placZ and pCD40L killed more than 50% of the P13.2 cells at effector:target ratios of 4:1. To achieve comparable levels of specific lysis required eight-fold higher effector:target ratios using splenocytes from mice that received intradermal injections of placZ and pCDNA3. Nevertheless, the splenocytes of mice co-injected with pCD40L and placZ did not have greater non-specific CTL activity for P815 cells than that of mice that received placZ along with pCDNA3 (Figure 20). As expected, the splenocytes from mice that received injections of pCDNA3 alone, or pCDNA3 and pCD40L, did not mediate specific lysis of P13.2 or P815 cells.

20

Table IV

Experiment #1      Injections of plasmid DNA i.m.: 4/3/96; 4/10/96; 4/17/96; 4/24/96

ELISA for anti-beta galactosidase

	Antibodies: Group	Animal	Dilution of Pre-Bleed (4/3)			Dilution of Bleed (4/17)		
			1/140	1/200	1/1000	1/140	1/200	1/1
5	pcDNA3 (p-control, 100 mcg) (Control vector)	1	0.09	0.11	0.09	0.06	0.06	
		2	0.11	0.09	0.09	0.07	0.07	
		3	0.12	0.11	0.10	0.09	0.09	
		4	0.11	0.10	0.10	0.08	0.11	
		<b>Avg.</b>	<b>0.11</b>	<b>0.11</b>	<b>0.11</b>	<b>0.11</b>	<b>0.11</b>	
		<b>S.D.</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.02</b>	
15	p-lacZ (50 mcg) + p-Control (50 mcg)	5	0.13	0.10	0.10	0.07	0.11	
		6	0.10	0.11	0.10	0.07	0.06	
		7	0.19	0.10	0.18	0.07	0.07	
		8	0.10	0.09	0.10	0.08	0.07	
		<b>Avg.</b>	<b>0.13</b>	<b>0.10</b>	<b>0.12</b>	<b>0.07</b>	<b>0.08</b>	
		<b>S.D.</b>	<b>0.04</b>	<b>0.01</b>	<b>0.04</b>	<b>0.01</b>	<b>0.02</b>	
20	p-lacZ (50 mcg) + pRcCMV-mCD40L (p-mCD40L, 50 mcg)	27	0.06	0.06	0.06	0.13	0.11	
		18	0.06	0.06	0.06	0.27	0.13	
		19	0.06	0.06	0.06	0.23	0.19	
		20	0.06	0.06	0.06	0.23	0.19	
		<b>Avg.</b>	<b>0.06</b>	<b>0.06</b>	<b>0.06</b>	<b>0.74</b>	<b>0.47</b>	
		<b>S.D.</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>1.06</b>	<b>0.66</b>	

Table V

Experiment #2                      Injections of plasmid DNA i.m.: 6/5/96; 6/12/96; 6/19/96; 6/26/96

Dilutions of sera for anti-beta

	Group	Animal	Dilution of Pre-Bleed (6/5)			Dilution of Bleed (7/1)		
			1/140	1/200	1/1000	1/140	1/200	1/1
5	galactosidase antibodies:							
	p-Control (50 mcg)	9	0.02	0.02	0.06	0.04	0.01	
	+	10	0.06	0.02	0.10	0.02	0.02	
10	p-mCD40L (50 mcg)	11	0.02	0.02	0.07	0.03	0.01	
		12	0.06	0.03	0.05	0.18	0.04	
		<b>Avg.</b>	<b>0.04</b>	<b>0.04</b>	<b>0.04</b>	<b>0.04</b>	<b>0.04</b>	
		<b>S.D.</b>	0.02	0.01	0.02	0.07	0.01	
15	p-lacZ (50 mcg)	5	0.02	0.03	0.02	0.06	0.04	
	+	6	0.03	0.02	0.03	0.14	0.03	
	p-Control (50 mcg)	7	0.56	0.13	0.06	0.29	0.06	
		8	0.01	0.02	0.05	0.06	0.02	
		<b>Avg.</b>	<b>0.15</b>	<b>0.05</b>	<b>0.04</b>	<b>0.13</b>	<b>0.04</b>	
		<b>S.D.</b>	0.27	0.05	0.02	0.11	0.02	
20	p-lacZ (50 mcg)	13	0.23	0.06	0.05	0.28	0.07	
	+	14	0.02	0.02	0.03	0.04	0.02	
	p-mCD40L (50 mcg)	15	0.02	0.02	0.02	0.89	0.21	
		16	0.05	0.04	0.02	0.11	0.04	
25		<b>Avg.</b>	<b>0.08</b>	<b>0.04</b>	<b>0.03</b>	<b>0.33</b>	<b>0.08</b>	
		<b>S.D.</b>	0.10	0.02	0.02	0.39	0.09	

Table VI

## Summary

		Pre-Immune @ beta-gal			Early @ beta-ga		
		1/140	1/200	1/1000	1/140	1/200	1/1
5	1) p-Control (n = 4)	Avg. 0.11	0.11	0.11	0.11	0.11	
		S.D. 0.01	0.01	0.01	0.01	0.02	
10	2) p-mCD40L + p-Control (n = 4)	Avg. 0.04	0.04	0.04	0.04	0.04	
		S.D. 0.02	0.01	0.02	0.07	0.01	
	3) p-lacZ + p-Control (n = 8)	Avg. 0.11	0.04	0.04	0.11	0.03	
		S.D. 0.22	0.04	0.01	0.09	0.02	
15	4) p-lacZ + p-mCD40L (n = 8)	Avg. 0.11	0.04	0.03	0.25	0.06	
		S.D. 0.10	0.02	0.01	0.32	0.07	
Anti-beta-galactosidase standard:							
20	O.D.	67 ng	22ng	7.4ng	2.5ng	.82ng	.27ng
		3.01	2.98	2.05	1.10	0.52	0.26
		3.14	3.14	2.25	1.20	0.56	0.26

Table VII

## BONFERRONI t-TESTS

5	Comparison	Difference of means			t	P<.05
	4 vs 2:	2.06 -	0.04 =	2.02	3.782	Yes
	4 vs 1:	2.06 -	0.11 =	1.95	3.651	Yes
	4 vs 3:	2.06 -	0.61 =	1.45	3.325	Yes
	3 vs 2:	0.61 -	0.04 =	0.57	1.067	No
10	3 vs 1:	0.61 -	0.11 =	0.50	Do not test	
	1 vs 2:	0.11 -	0.04 =	0.07	Do not test	

Degrees of freedom: 20

15

## ONE WAY ANALYSIS OF VARIANCE

	Group	N	Mean	Std Dev	SEM
	1	4	0.11	0.01	0.00
20	2	4	0.04	0.04	0.02
	3	8	0.61	1.11	0.39
	4	8	2.06	0.97	0.34
	5	4	1.51	0.77	0.38
	6	4	1.14	0.53	0.26
25	7	4	0.83	0.43	0.22

## ONE WAY ANALYSIS OF VARIANCE

	Source of Variation	SS	DF	Variance Est (MS)
30	Between Groups	18.29	6	3.05
	Within Groups	18.39	29	0.63
	Total	36.69	35	

$$35 \quad F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{Mswit} = \frac{3.05}{0.63} = 4.81 \quad P = 0.002$$



4. Treatment of Neoplasia Using a Gene Therapy Vector Containing an Accessory Molecule Gene or Chimeric Accessory Molecule Gene

5 a. Treatment of Neoplasia in Mice

The treatment of a neoplasia in a mouse model system has been demonstrated using the genes encoding accessory molecule ligands of the present invention. Gene therapy vectors containing an accessory molecule ligand gene (murine CD40 ligand) were prepared as has been previously described in the above examples. These gene therapy vectors were used to introduce that accessory molecule ligand gene into neoplastic cells, Linel cells, from a tumor which originated in BALB/c mice. The accessory molecules were introduced into the neoplastic cells according to the above examples. The expression of the accessory molecule ligand on the surface of these neoplastic cells was confirmed using flow cytometry as has been described in the above examples.

The effectiveness of the accessory molecule ligand genes for treating neoplasia was shown as follows. Female BALB/c mice (6-8 weeks old) were injected i.p. with  $1.0 \times 10^5$  irradiated Linel neoplastic cells. The neoplastic Linel cells are derived from a spontaneous lung adenocarcinoma in a BALB/c mouse. This neoplastic cell has been described by Blieden et al., Int. J. Cancer Supp., 6:82 (1991). Other female BALB/c mice were injected i.p. with  $1.0 \times 10^5$  irradiated Linel tumor cells that had previously been transduced with the gene therapy vector encoding the accessory molecule ligand gene (murine CD40) as described above.

Each group of mice was allowed to generate an immune response for 10 days. After 10 days each mouse

was challenged with  $1.0 \times 10^4$  live, non-irradiated Line1 neoplastic cells. These mice were then monitored for the formation of tumors and then sacrificed when the tumors grew to 2.0 cm because of morbidity. The results of this monitoring are shown in Figure 7. As can be seen by Figure 7, the mice immunized with the neoplastic cell expressing the accessory molecule ligands of the present invention on the cell surface remained free of tumor throughout the experiment. Mice immunized with the neoplastic cells not having the accessory molecule ligand genes of the present invention succumbed to tumor 50 days after challenge with the neoplastic cells.

Figure 21 demonstrates downmodulation of human CD40L, but not murine CD40L, in lung tumor cell lines that express CD40. Human cell lines HeLa (CD40-negative cervical carcinoma, Figure 21A), A427 (CD40-negative lung carcinoma, Figure 21B), NCI 460 (weakly CD40-positive lung large cell carcinoma, Figure 21C), and SK-Mes-1 (strongly CD40-positive lung squamous cell tumor, Figure 21D) were infected with adenovirus encoding lac-Z (Ad-LacZ), murine CD40L (Ad-mCD40L), and human CD40L (Ad-hCD40L) at an MOI of 0 (Blank), 1, and 10. 48 hours after infection, murine CD40L and human CD40L surface expression was determined. The percentage of cells that express ligand are plotted on the Y-axis. Human and mouse CD40L are expressed at equal levels in CD40-negative cell lines. However, only murine CD40L expression is stable on cell lines that express CD40. In contrast to mCD40L, human CD40L is downmodulated on CD40-positive tumors.

The data graphed in Figure 22A show that CD40 binding induces expression of tumor surface markers. Treating CD40-expressing lung cancer cell lines with  $\alpha$ CD40 mAb resulted in enhanced expression of the tumor cell surface markers CD95 (Fas), CD54 (ICAM-1) and class

I major histocompatibility antigens (MHC I). NCI 460, a weakly CD40-positive lung large cell carcinoma, was incubated with a CD40-specific monoclonal antibody (thick line), or MOPC21, an isotype control mAb (thin line), on CD32-expressing mouse fibroblasts for 48 hours. Following the 48 hr incubation, the lung tumor cells were analyzed for CD95, CD54, and MHC-I expression by FACS.

Figure 22B again shows downmodulation of human CD40L by CD40-positive tumor cells. HeLa (CD40-negative), CLL (CD40-positive), and SK-MES-1 (CD40-positive) tumor cells were cocultured for 24 hours with CD3-activated normal donor T cells at a tumor cell:T cell ration of 2.5:1. Following coculture, CD2-expressing T cells were analyzed for CD40L surface expression by FACS. Thin lines represent T cells stained with FITC-labeled isotype control antibody (MOPC21) and thick lines represent activated T cells stained with FITC-labeled  $\alpha$ CD40L antibody ( $\alpha$ CD154 antibody). The CD40-positive tumor cell lines, SK-MES-1, and CLL, do not express CD40 ligand on their surfaces.

5. Expression of the Human and Mouse Accessory Molecule Ligand, Fas Ligand, in Human Blood Lymphocytes

a. Construction of a Genetic Construct and Gene Therapy Vector Containing the Human and Mouse Fas Ligand Gene

Either the human accessory molecule ligand gene (human Fas ligand) or the murine accessory molecule ligand gene (murine Fas ligand) was constructed utilizing the respective human and murine genes.

An altered accessory cell molecule, in which a putative MMP-cleavage site was removed, was made and designated  $\Delta$ FasL-pcDNA3. The nucleotide sequence of  $\Delta$ FasL-pcDNA3 is listed as SEQ ID NO: 40. Human Fas ligand nucleotides 325 to 342, encoding six amino acids, are missing from  $\Delta$ FasL. The design of  $\Delta$ FasL was based on reasoning that Domain III contains sites most accessible to MMPs, and could thus be the target on the molecule for cleavage from the surface of the cell. Sequences of the human Fas ligand gene have been determined and are listed as SEQ ID NOS: 13 and 30 (Genbank accession U11821). Sequences of mouse Fas ligand genes have been determined and are listed as SEQ ID NOS: 14 (C57BL/6, Genbank accession U10984) and 31 (Balb/c, Genbank accession U58995). The sequence of the rat Fas ligand gene has been determined and is listed as SEQ ID NO: 25 (Genbank accession U03470). Chimeric constructs are made, as described in Example 2 for CD40 ligand chimeric constructs, in which Domain III of human Fas ligand is replaced with Domains of other proteins, particularly proteins of the TNF family. Chimeric constructs include, but are not limited to, human Fas ligand with Domain III replaced by Domain III of murine Fas ligand (chimeric sequence listed as SEQ ID NO: 37, sequence line-up shown in Figure 37), or replaced by Domain III of human CD70 (chimeric sequence listed as SEQ ID NO: 38, sequence line-up shown in Figure 38), or replaced with Domain I of human CD70 (chimeric sequence listed as SEQ ID NO: 39, sequence line-up shown in Figure 39). Chimeric constructs in which multiple domains, for example, two copies of human CD70 Domain III, are inserted into human Fas ligand in place of Domain III, are also made using methods described in Example I. Chimeric constructs in which synthetic sequences are

used to replace Domain III of human Fas ligand are also made.

i. Human Fas Ligand Cloning

5 The cDNA encoding human Fas-ligand was subcloned in the eukaryotic expression vector pcDNA3. Normal donor blood lymphocytes were activated for 4 hours with 1 ng/ml PMA plus 0.5 uM ionomycin. Total RNA was isolated  
10 with the Qiagen Rneasy kit. cDNA was then synthesized from poly-A RNA with oligo-dT primers using the Gibco-BRL Superscript cDNA synthesis kit. The gene encoding human Fas-ligand was then PCR amplified with the Fas-ligand-specific primers (sense primer, SEQ ID NO: 32,  
15 antisense primer, SEQ ID NO: 33). The Fas-ligand PCR product was then subcloned into pcDNA3 using standard molecular biology techniques. RT-PCR products, subcloned into pcDNA3, are designated hFasL-pcDNA3.

20 ii. Murine Fas Ligand Cloning

The murine Fas-ligand genes from Balb/c and C57/BL6 strains of mice were also amplified following activation of mouse splenocytes with PMA plus ionomycin as  
25 described above, and amplified from poly-A synthesized cDNA as described above (sense primer, SEQ ID NO: 34, antisense primer, SEQ ID NO: 35). These genes were subcloned in the pTARGET expression vector (Promega, Madison, WI). RT-PCR products, subcloned into pcDNA3,  
30 are designated mFasL-pcDNA3.

iii. Adenovirus Vector Construction

For construction of adenovirus vectors encoding  
35 human Fas-ligand, murine Fas-ligand or  $\Delta$ Fas-ligand, the

cloned cDNA insert is subcloned into the plasmid pRc/RSV (Invitrogen, San Diego, CA) at the HindIII-XbaI site. A BglIII-XhoI fragment with the RSV promoter-enhancer and the bovine growth hormone poly-A signal sequence was subcloned into the BamHI-XhoI site of plasmid MCS(SK)pXCX2. The plasmid MCS(SK)pXCX2 is a modification of the plasmid pXCX2, in which the pBluescript polylinker sequence was cloned into the E1 region. The resulting plasmid then is co-transfected along with pJM17 into 293 cells using the calcium phosphate method. Isolated plaques of adenovirus vectors are picked and expanded by infecting 293 cells. High titer adenovirus preparations are obtained, as described above which uses a cesium chloride gradient for concentrating virus particles via a step gradient, with the densities of 1.45g/cm<sup>3</sup> and 1.20g/cm<sup>3</sup>, in which samples are centrifuged for 2 hours in an SW41 rotor (Beckman, Brea, CA) at 25,000 rpm at 4° C. The virus band is desalted using a Sephadex G-25 DNA grade column (Pharmacia, Piscataway, NJ), and the isolated virus is stored at -70° C in phosphate-buffered saline with 10% glycerol. The titer of the virus is determined by infecting permissive 293 cells at various dilutions and counting the number of plaques. Titers typically range from 10<sup>10</sup> to 10<sup>12</sup> plaque forming units/ml. The adenovirus constructs are designated Ad-hFasL, Ad-mFasL and Ad-ΔFasL.

b. Introduction of the Murine and Human Fas Ligand Genes into Human Cells

The constructs hFasL-pcDNA3, mFasL-pcDNA3 and ΔFasL-pcDNA3 are transfected into 293 via electroporation. The transfected cells are selected in medium containing G418. Fas-ligand transfectants are screened for expression of the transgene using anti-Fas-ligand antibody and flow

cytometry. The methods used are similar to those described for transfection of CD40L into CLL cells.

For FasL-adenovirus infection,  $10^6$  freshly thawed and washed CLL cells or HeLa cells are suspended in 0.5 to 1 mL of culture medium for culture at 37°C in a 5% CO<sub>2</sub>-in-air incubator. Adenovirus are added to the cells at varying multiplicity of infection (MOI), and the infected cells are cultured for 48 hours, unless otherwise stated, before being analyzed for transgene expression.

c. Expression of the Fas Ligand Genes in Human Cells

Mice with the lymphoproliferative or generalized lymphoproliferative disorder are unable to delete activated self-reactive cells outside of the thymus. This is related to the fact that, in these mice, interactions between the Fas receptor and an accessory molecule ligand, Fas ligand, are defective. These animals develop numerous disorders including lymphadenopathy, splenomegaly, nephritis, and systemic autoimmune pathology which resembles that seen in patients with systemic lupus erythematosus or rheumatoid arthritis (RA). It is conceivable that the normal interactions between the Fas receptor and the accessory molecule ligand that are responsible for clearance of activated lymphocytes from joints may be impaired in RA patients.

RA synovial lymphocytes express the Fas receptor at a higher proportion than that of matched RA blood lymphocytes to matched normal donor blood lymphocytes. On the other hand, RA synovial lymphocytes express little or no accessory molecule ligand. Since the RA synovial lymphocytes are sensitive to Fas-induced apoptosis, it is feasible that local expression of Fas ligand in the RA

joint could serve to eliminate the synovial mononuclear cells that potentially mediate RA autoimmune pathology.

Figure 23 shows that Fas-ligand expression in lymphocytes is inhibited by exposure to RA synovial fluid. Normal donor blood T cells were activated for 5 hours with 1 ng/ml PMA plus 0.5  $\mu$ M ionomycin. Cells were incubated in the presence of rheumatoid arthritis blood plasma (circles), RA synovial fluid (diamonds), or neither (squares). In addition, cells were incubated with increasing concentrations of the MMP inhibitor BB94. Following activation, cells were analyzed for Fas-ligand surface expression by FACS. The percentage of cells expressing Fas ligand are plotted in Figure 23. This experiment demonstrates that there is a factor(s) present in RA synovial fluid and serum that prevents surface expression of Fas-ligand.

d. Function of Human, Murine and Chimeric  
Accessory Molecule Ligand, Fas Ligand

To determine the capacity of the  $\Delta$ FasL constructs, the above-mentioned transfected cells are mixed with the Fas-ligand sensitive human T cell line, JURKAT. Following 4 hours coculture, the nonadherent JURKAT cells are collected and evaluated for apoptosis. The fluorescent compound 3,3' dihexyloxacarbocyanine iodide (DiOC<sub>6</sub>) is used to evaluate for apoptosis using a modification of a previously described protocol. For this, the cells are washed once at room temperature in phosphate buffered saline (PBS, pH 7.2). Cells are placed into separate wells of a 96 well U-bottom plastic microtiter plate at  $10^5$  -  $5 \times 10^5$  cells/well in 50 ml total volume. If indicated, saturating amounts of PE-conjugated antibodies are added followed by addition of



DiOC<sub>6</sub> and propidium iodide (PI). DiOC<sub>6</sub> and PI are used at 40 nM and 10 ng/ml final concentrations, respectively. The cells are then incubated 15 minutes in a 37°C, 5% CO<sub>2</sub> tissue culture incubator. The stained cells are then washed twice in ice cold PBS and ultimately suspended in 200 ml SM and analyzed by FACS. Dead cells and debris with characteristic forward and light scatter profiles and PI staining are excluded from analysis.

The ability of cells expressing ΔFasL-pcDNA3 to direct Fas-mediated apoptosis of cells expressing CD95 is compared with that of cells expressing FasL-pcDNA3. Relative stability of the protein products encoded by ΔFasL-pcDNA3 or FasL-pcDNA3 pre- and post- culture with RA synovial fluid, and with or without the metalloproteinase inhibitors, are assessed via flow cytometry of cells expressing either ligand.

#### 6. Treatment of Arthritis with Gene Therapy Vectors Encoding an Accessory Molecule Ligand, Fas Ligand

The heterologous Fas-ligand constructs, made as described above, that show the highest stability of expression in combination with the greatest ability to mediate Fas-induced apoptosis, are used in gene therapy for RA. Potential therapeutic constructs are tested in well-characterized mouse models of arthritis to assess efficacy and function *in vivo*.

##### a. Gene Therapy Treatment of Arthritis in Mice

##### i. Mouse Models for Arthritis

One mouse arthritis model is collagen-induced arthritis. It is known that injecting DBA/1 mice with type II collagen in complete Freund's adjuvant (CFA)

induces an arthritis with synovitis and erosions that histologically resemble RA. For our studies, male DBA/I mice are immunized with bovine type II collagen in complete Freund's adjuvant on day 0 and boosted  
5 intraperitoneally (i.p.) on day 21. On day 28, animals are given an additional i.p. injection with lipopolysaccharide (LPS) and/or the same type collagen, or an injection of acetic acid alone. Swelling and/or redness of a fore or hind paw in animals immunized with  
10 collagen typically is detected the third or fourth week following the second injection. The vertebrae are only rarely affected, and then only weeks after the initial peripheral joint swelling. Affected joints display initial histologic changes of synovial edema, followed by  
15 synovial hyperplasia.

Another animal model, recently described by Kouskoff, V. et al., in Cell 87:811-822 (1997) was generated fortuitously, by crossing a T cell receptor (TCR) transgenic mouse line with the non-obese-diabetic  
20 (NOD) strain to produce the KRN x NOD mouse model of RA. The offspring of such a mating universally develop a joint disease that is highly similar to that of patients with RA. Moreover, the disease in these animals has an early and reproducible time of onset and a highly  
25 reproducible course. The arthritis apparently is induced by chance recognition of an NOD-derived major histocompatibility complex (MHC) class II molecule by the transgenic TCR, leading to breakdown in the general mechanisms of self-tolerance and systemic self-  
30 reactivity.

ii. Relief of Arthritis Symptoms in Mice  
Treated with a Gene Therapy Vector  
Encoding an Accessory Molecule Ligand

5 We have adapted and modified a protocol originally  
described by Sawchuk and colleagues for micro-injecting  
adenovirus vectors into mouse joints. Using this  
procedure we can reproducibly inject a 5  $\mu$ l volume into  
the articular space of the mouse knee. In this  
10 procedure, the mice are anesthetized with metofane. A  
small incision of approximately 2-3 mm is made with a #11  
scalpel blade in the skin over the lateral aspect of the  
knee to visualize the patello-tibial ligament. We can  
inject up to 5  $\mu$ l of fluid using a micro-100  $\mu$ l-Hamilton  
15 syringe and a 30-gauge needle. After the injection, the  
knee incision is closed with Nexabond (Veterinary  
Products Laboratory). Our adenovirus titers typically  
exceed  $10^{10}$  plaque forming units (pfu) per ml, making it  
possible to deliver at least  $5 \times 10^8$  pfu of virus in 5 ml  
20 into the knee joints, as outlined above. Control animals  
are injected with control Ad-lacZ vector, a replication-  
defective adenovirus vector lacking a transgene, or with  
the buffer used to suspend the virus (10 mM Tris, 1 mM  
MgCl<sub>2</sub>, 10% glycerol).

25 In another method, splenocytes will be harvested  
from mice that are syngeneic to the host animal intended  
for adoptive transfer of transduced cells. Cell  
proliferation will be induced with exogenous IL-12 (100  
units/ml) for 48 h. Cells are counted and then re-plated  
30 at densities of  $5 \times 10^5$  or  $1 \times 10^6$  cells per ml in a 12-  
well dish with 1 ml complete culture medium per well.  
Virus and ConA are added together at the time of re-  
plating in the presence of polybrene (8  $\mu$ g/ml). The  
medium is changed 24 hours after infection with complete  
35 medium containing 100 units of recombinant IL-2 per ml.

Aliquots of the transduced cells are examined, for Fas-ligand expression, at 48 hours after infection via flow cytometry.

Animals will receive standardized numbers of  
5 cytokine-producing cells or control mock-transfected cells intraperitoneally. Concentrated cell suspensions are injected directly into the mouse synovium, as described in section 4A above. In parallel, aliquots of the transferred cell populations are maintained in tissue  
10 culture supplemented with exogenous IL-2.

Mice are monitored in a blinded fashion for signs of arthritis. The date of disease onset is recorded and clinical severity of each joint or group of joints (toes, tarsus, ankle, wrist, knee) are graded as follows: 0  
15 (normal), 1 (erythema), 2 (swelling), 3 (deformity), 4 (necrosis). The scores are summed to yield the arthritic score. The severity of arthritis is expressed both as the mean score observed on a given day, and as the mean of the maximal arthritic score reached by each mouse  
20 during the clinical course of the disease. At the time of death, hind paws are dissected free and processed for histologic examination or for RT-PCR. The histologic severity of the arthritis is scored on a scale of 0-3 for synovial proliferation and inflammatory cell  
25 infiltration, where a score of 0 = normal and 3 = severe.

For mice receiving intra-synovial injection of control of test adenovirus vector, the level of arthritis observed between contralateral sites is compared. In addition, the overall joint score minus that of the  
30 injected joint for the entire animal is compared with that observed in the joint injected with the control or test adenovirus vector.

Local administration of Fas-ligand adenovirus expression vectors will result in clearance of activated  
35 cells, as assessed by measuring the relative levels of

CD80 mRNA by quantitative RT-PCR. This treatment also will lead to an enhanced level. Also, whether such level of apoptosis identified in affected mouse synovial tissue is assessed by the TUNEL assay ("Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP Nick End Labeling"). TUNEL is performed by immersing the sections in TdT buffer (30 mM Tris-HCl, pH 7.2, 140 nM sodium cacodylate, 1 mM cobalt chloride), and then adding TdT (GIBCO BRL, Grand Island, NY) and biotinylated dUTP (Boehringer Mannheim, Indianapolis, IN). The reaction is terminated by immersing the sections in TB buffer (300 mM sodium chloride, 30 mM sodium citrate). Subsequently, the samples are treated with peroxidase-labeled streptavidin and then visualized using the VECTASTAIN ABC kit (Vector Laboratories Inc., Burlingame, CA). For immunohistochemistry, the sections are blocked with 4% skim milk for 30 minutes at room temperature, then incubated with biotinylated mAbs specific for mouse CD3, B220, CD80, or CD95 (Fas). These antibodies are available from Pharmingen (San Diego, CA).

b. Treatment of Rheumatoid Arthritis Patients with a Gene Therapy Vector Encoding an Accessory Molecule Ligand, Fas Ligand

25

Candidate Fas-ligand constructs identified as having potential therapeutic benefit are used in human protocols to treat RA. Human protocols encompass either *in vivo* or *ex vivo* methods to deliver the Fas-ligand constructs.

Furthermore, the Fas-ligand constructs are potentially delivered by either viral or non-viral methods. Outlines of therapeutic strategies are described below.

An *ex vivo* therapy is similar to a protocol described for intra-articular transplantation of autologous synoviocytes retrovirally transduced to

synthesize interleukin-1 receptor antagonist (Evan, Christopher et. al., Clinical Trial to Assess the Safety, Feasibility, and Efficacy of Transferring a Potentially Anti-Arthritic Cytokine Gene to Human Joints with

5 Rheumatoid arhtritis, Human Gene Therapy, Vol. 7, 1261-1280). In this procedure, after clinical diagnosis of RA, the synovium is harvested during total joint replacement. The synoviocytes re-isolated and expanded, then transduced or transfected with heterologous Fas-

10 ligand into synoviocytes (via retrovirus, adenovirus, naked DNA, etc.). The gene-modified synoviocytes are then reinjected into the patient, who is monitored and tested for amelioration of RA-associated symptoms, and for expression and function of the Fas-ligand in modified

15 synoviocytes.

In another ex vivo protocol, an allogeneic immortalized cell line that stably expresses the heterologous Fas-ligand is administered to the RA patient. In this protocol, a stable immortalized cell

20 line expressing Fas-ligand (introduced by transfection of the gene into the cell by nonviral methods, such as electroporation), or by viral transduction of the gene into the cell) is constructed. The modified cell line is injected into the patient, who is monitored and tested

25 for amelioration of RA associated symptoms, and for expression and function of the hFas-ligand in modified synoviocytes.

An in vivo based therapy will is similar in concept to the amelioration of collagen-induced-arthritis using a murine Fas-ligand adenovirus gene therapy vector,

30 described in Zhang, et al., J. Clin. Invest. 100:1951-1957 (1997). In our use of such an approach, delivery of the hFas-ligand construct or chimeric  $\Delta$ fasL directly to the joints of RA patients is performed using either viral

35 or non-viral methods. In this procedure, the Fas-ligand

construct (e.g. hFas-ligand adenovirus) is directly injected into the synovium. Patients are monitored and tested for amelioration of RA-associated symptoms as well as biological testing for expression and function of the

5 hFas-ligand in modified synoviocytes.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANTS: Kipps, Thomas J.  
Sharma, Sanjai  
Cantwell, Mark

(ii) TITLE OF INVENTION: NOVEL EXPRESSION VECTORS  
CONTAINING ACCESSORY  
MOLECULE LIGAND GENES AND  
THEIR USE FOR IMMUNOMODULA-  
TION AND TREATMENT OF  
MALIGNANCIES AND AUTOIMMUNE  
DISEASE

(iii) NUMBER OF SEQUENCES: 35

## (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Lyon & Lyon  
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Suite 4700  
(C) CITY: Los Angeles  
(D) STATE: California  
(E) COUNTRY: U.S.A.  
(F) ZIP: 90071-2066

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette,  
1.44 Mb storage  
(B) COMPUTER: IBM Compatible  
(C) OPERATING SYSTEM: IBM P.C. DOS 5.0  
(D) SOFTWARE: FastSeq Version 2.0

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: To Be Assigned  
(B) FILING DATE:  
(C) CLASSIFICATION:



## (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 60/132145  
(B) FILING DATE: 12/9/96

## (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Guise, Jeffrey W.  
(B) REGISTRATION NUMBER: 34,613  
(C) REFERENCE/DOCKET NUMBER: 231/003

## (ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (213) 489-1600  
(B) TELEFAX: (213) 955-0440  
(C) TELEX: 67-3510

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 786 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATGATCGAAA	CATACAACCA	AACTTCTCCC	CGATCTGCGG	CCACTGGACT	GCCCATCAGC	60
ATGAAAATTT	TTATGTATTT	ACTTACTGTT	TTTCTTATCA	CCCAGATGAT	TGGGTCAGCA	120
CTTTTTGCTG	TGTATCTTCA	TAGAAGGTTG	GACAAGATAG	AAGATGAAAG	GAATCTTCAT	180
GAAGATTTTG	TATTCATGAA	AACGATACAG	AGATGCAACA	CAGGAGAAAG	ATCCTTATCC	240
TTACTGAACT	GTGAGGAGAT	TAAAAGCCAG	TTTGAAGGCT	TTGTGAAGGA	TATAATGTTA	300
AACAAAGAGG	AGACGAAGAA	AGAAAACAGC	TTTGAAATGC	AAAAAGGTGA	TCAGAATCCT	360
CAAATTGCGG	CACATGTCAT	AAGTGAGGCC	AGCAGTAAAA	CAACATCTGT	GTTACAGTGG	420
GCTGAAAAAG	GATACTACAC	CATGAGCAAC	AACTTGGTAA	CCCTGGAAAA	TGGGAAACAG	480
CTGACCGTTA	AAAGACAAGG	ACTCTATTAT	ATCTATGCCC	AAGTCACCTT	CTGTTCCAAT	540
CGGGAAGCTT	CGAGTCAAGC	TCCATTTATA	GCCAGCCTCT	GCCTAAAGTC	CCCCGGTAGA	600
TTCGAGAGAA	TCTTACTCAG	AGCTGCAAAT	ACCCACAGTT	CCGCCAAACC	TTGCGGGCAA	660
CAATCCATTC	ACTTGGGAGG	AGTATTTGAA	TTGCAACCAG	GTGCTTCGGT	GTTTGTCAAT	720
GTGACTGATC	CAAGCCAAGT	GAGCCATGGC	ACTGGCTTCA	CGTCCTTTGG	CTTACTCAAA	780
CTCTGA						786

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 783 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATGATAGAAA	CATACAGCCA	ACCTTCCCCC	AGATCCGTGG	CAACTGGACT	TCCAGCGAGC	60
ATGAAGATTT	TTATGTATTT	ACTTACTGTT	TTCTTATCA	CCCAAATGAT	TGGATCTGTG	120
CTTTTGTCTG	TGTATCTTCA	TAGAAGATTG	GATAAGGTCG	AAGAGGAAGT	AAACCTTCAT	180
GAAGATTTTG	TATTCATAAA	AAAGCTAAAG	AGATGCAACA	AAGGAGAAGG	ATCTTTATCC	240
TTGCTGAACT	GTGAGGAGAT	GAGAAGGCAA	TTTGAAGACC	TTGTCAAGGA	TATAACGTTA	300
AACAAAGAAG	AGAAAAAAGA	AAACAGCTTT	GAAATGCAAA	GAGGTGATGA	GGATCCTCAA	360
ATTGCAGCAC	ACGTTGTAAG	CGAAGCCAAC	AGTAATGCAG	CATCCGTTCT	ACAGTGGGCC	420
AAGAAAGGAT	ATTATACCAT	GAAAAGCAAC	TTGGTAATGC	TTGAAAATGG	GAAACAGCTG	480
ACGGTTAAAA	GAGAAGGACT	CTATTATGTC	TACACTCAAG	TCACCTTCTG	CTCTAATCGG	540
GAGCCTTCGA	GTCAACGCCC	ATTATCGTC	GGCCTCTGGC	TGAAGCCCAG	CATTGGATCT	600
GAGAGAATCT	TACTCAAGGC	GGCAAATACC	CACAGTTCCT	CCCAGCTTTG	CGAGCAGCAG	660
TCTGTTCACT	TGGGCGGAGT	GTTTGAATTA	CAAGCTGGTG	CTTCTGTGTT	TGTCAACGTG	720
ACTGAAGCAA	GCCAAGTGAT	CCACAGAGTT	GGCTTCTCAT	CTTTTGGCTT	ACTCAAATC	780
TGA						783

## (2) INFORMATION FOR SEQ ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 783 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGATCGAAA	CATACAACCA	AACTTCTCCC	CGATCTGCGG	CCACTGGACT	GCCCATCAGC	60
ATGAAAATTT	TTATGTATTT	ACTTACTGTT	TTTCTTATCA	CCCAGATGAT	TGGGTCAGCA	120
CTTTTGTCTG	TGTATCTTCA	TAGAAGATTG	GATAAGGTCG	AAGAGGAAGT	AAACCTTCAT	180
GAAGATTTTG	TATTCATAAA	AAAGCTAAAG	AGATGCAACA	AAGGAGAAGG	ATCTTTATCC	240
TTGCTGAACT	GTGAGGAGAT	GAGAAGGCAA	TTTGAAGACC	TTGTCAAGGA	TATAACGTTA	300
AACAAAGAAG	AGAAAAAAGA	AAACAGCTTT	GAAATGCAAA	GAGGTGATGA	GGATCCTCAA	360
ATTGCAGCAC	ACGTTGTAAG	CGAAGCCAAC	AGTAATGCAG	CATCCGTTCT	ACAGTGGGCC	420
AAGAAAGGAT	ATTATACCAT	GAAAAGCAAC	TTGGTAATGC	TTGAAAATGG	GAAACAGCTG	480
ACGGTTAAAA	GAGAAGGACT	CTATTATGTC	TACACTCAAG	TCACCTTCTG	CTCTAATCGG	540
GAGCCTTCGA	GTCAACGCCC	ATTATCGTC	GGCCTCTGGC	TGAAGCCCAG	CATTGGATCT	600
GAGAGAATCT	TACTCAAGGC	GGCAAATACC	CACAGTTCCT	CCCAGCTTTG	CGAGCAGCAG	660

TCTGTTCACT	TGGGCGGAGT	GTTTGAATTA	CAAGCTGGTG	CTTCTGTGTT	TGTCAACGTG	720
ACTGAAGCAA	GCCAAGTGAT	CCACAGAGTT	GGCTTCTCAT	CTTTTGGCTT	ACTCAAACTC	780
TGA						783

## (2) INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

(A)	LENGTH:	786 base pairs
(B)	TYPE:	nucleic acid
(C)	STRANDEDNESS:	single
(D)	TOPOLOGY:	linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATGATCGAAA	CATACAACCA	AACTTCTCCC	CGATCTGCGG	CCACTGGACT	GCCCATCAGC	60
ATGAAAATTT	TTATGTATTT	ACTTACTGTT	TTCCTTATCA	CCCAAATGAT	TGGATCTGTG	120
CTTTTGGCTG	TGTATCTTCA	TAGAAGGTTG	GACAAGATAG	AAGATGAAAG	GAATCTTCAT	180
GAAGATTTTG	TATTCATGAA	AACGATACAG	AGATGCAACA	CAGGAGAAAG	ATCCTTATCC	240
TTACTGAACT	GTGAGGAGAT	TAAAAGCCAG	TTTGAAGGCT	TTGTGAAGGA	TATAATGTTA	300
AACAAAGAGG	AGACGAAGAA	AGAAAACAGC	TTTGAAATGC	AAAAAGGTGA	TCAGAATCCT	360
CAAATTGCGG	CACATGTCAT	AAGTGAGGCC	AGCAGTAAAA	CAACATCTGT	GTTACAGTGG	420
GCTGAAAAAG	GATACTACAC	CATGAGCAAC	AACTTGGTAA	CCCTGGAAAA	TGGGAAACAG	480
CTGACCGTTA	AAAGACAAGG	ACTCTATTAT	ATCTATGCCC	AAGTCACCTT	CTGTTCCAAT	540
CGGGAAGCTT	CGAGTCAAGC	TCCATTTATA	GCCAGCCTCT	GCCTAAAGTC	CCCCGGTAGA	600
TTGAGAGAGAA	TCTTACTCAG	AGCTGCAAAT	ACCCACAGTT	CCGCCAAACC	TTGCGGGCAA	660
CAATCCATTC	ACTTGGGAGG	AGTATTTGAA	TTGCAACCAG	GTGCTTCGGT	GTTTGTCAAT	720
GTGACTGATC	CAAGCCAAGT	GAGCCATGGC	ACTGGCTTCA	CGTCCTTTGG	CTTACTCAAA	780
CTCTGA						786

## (2) INFORMATION FOR SEQ ID NO: 5:

## (i) SEQUENCE CHARACTERISTICS:

(A)	LENGTH:	783 base pairs
(B)	TYPE:	nucleic acid
(C)	STRANDEDNESS:	single
(D)	TOPOLOGY:	linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ATGATCGAAA	CATACAACCA	AACTTCTCCC	CGATCTGCGG	CCACTGGACT	GCCCATCAGC	60
ATGAAAATTT	TTATGTATTT	ACTTACTGTT	TTCCTTATCA	CCCAAATGAT	TGGATCTGTG	120
CTTTTGGCTG	TGTATCTTCA	TAGAAGATTG	GATAAGGTCG	AAGAGGAAGT	AAACCTTCAT	180
GAAGATTTTG	TATTCATAAA	AAAGCTAAAG	AGATGCAACA	AAGGAGAAGG	ATCTTTATCC	240

[illegible]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 786 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

ATGATAGAAA	CATACAGCCA	ACCTTCCCCC	AGATCCGTGG	CAACTGGACT	TCCAGCGAGC	60
ATGAAGATTT	TTATGTATTT	ACTTACTGTT	TTTCTTATCA	CCCAGATGAT	TGGGTCAGCA	120
CTTTTTTGCTG	TGTATCTTCA	TAGAAGGTTG	GACAAGATAG	AAGATGAAAG	GAATCTTCAT	180
GAAGATTTTG	TATTCATGAA	AACGATACAG	AGATGCAACA	CAGGAGAAAAG	ATCCTTATCC	240
TTACTGAACT	GTGAGGAGAT	TAAAAGCCAG	TTTGAAGGCT	TTGTGAAGGA	TATAATGTTA	300
AACAAAGAGG	AGACGAAGAA	AGAAAACAGC	TTTGAAATGC	AAAAAGGTGA	TCAGAATCCT	360
CAAATTGCGG	CACATGTCAT	AAGTGAGGCC	AGCAGTAAAA	CAACATCTGT	GTTACAGTGG	420
GCTGAAAAAG	GATACTACAC	CATGAGCAAC	AACTTGGTAA	CCCTGGAAAA	TGGGAAACAG	480
CTGACCGTTA	AAAGACAAGG	ACTCTATTAT	ATCTATGCCC	AAGTCACCTT	CTGTTCCAAT	540
CGGGAAGCTT	CGAGTCAAGC	TCCATTTATA	GCCAGCCTCT	GCCTAAAAGTC	CCCCGGTAGA	600
TTCGAGAGAA	TCTTACTCAG	AGCTGCAAAT	ACCCACAGTT	CCGCCAAACC	TTGCGGGCAA	660
CAATCCATTTC	ACTTGGGAGG	AGTATTTGAA	TTGCAACCAG	GTGCTTCGGT	GTTTGTCAAT	720
GTGACTGATC	CAAGCCAAGT	GAGCCATGGC	ACTGGCTTCA	CGTCCTTTGG	CTTACTCAA	780
CTCTGA						788

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 786 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ATGATAGAAA	CATACAGCCA	ACCTTCCCCC	AGATCCGTGG	CAACTGGACT	TCCAGCGAGC	60
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CTTTTTGCTG	TGTATCTTCA	TAGAAGGTTG	GACAAGATAG	AAGATGAAAG	GAATCTTCAT	180
GAAGATTTTG	TATTCATGAA	AACGATACAG	AGATGCAACA	CAGGAGAAAG	ATCCTTATCC	240
TTACTGAACT	GTGAGGAGAT	TAAAAGCCAG	TTTGAAGGCT	TTGTGAAGGA	TATAATGTTA	300
AACAAAGAGG	AGACGAAGAA	AGAAAACAGC	TTTGAATGTC	AAAAAGGTGA	TCAGAATCCT	360
CAAATTGCGG	CACATGTCAT	AAGTGAGGCC	AGCAGTAAAA	CAACATCTGT	GTTACAGTGG	420
GCTGAAAAAG	GATACTACAC	CATGAGCAAC	AACTTGGTAA	CCCTGGAAAA	TGGGAAACAG	480
CTGACCGTTA	AAAGACAAGG	ACTCTATTAT	ATCTATGCCC	AAGTCACCTT	CTGTTCCAAT	540
CGGGAAGCTT	CGAGTCAAGC	TCCATTTATA	GCCAGCCTCT	GCCTAAAGTC	CCCCGGTAGA	600
TTCGAGAGAA	TCTTACTCAG	AGCTGCAAAT	ACCCACAGTT	CCGCCAAACC	TTGCGGGCAA	660
CAATCCATTC	ACTTGGGAGG	AGTATTTGAA	TTGCAACCAG	GTGCTTCGGT	GTTTGTCAAT	720
GTGACTGATC	CAAGCCAAGT	GAGCCATGGC	ACTGGCTTCA	CGTCCTTTGG	CTTACTCAAA	780
CTCTGA						786

## (2) INFORMATION FOR SEQ ID NO: 8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 864 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AACTCTAACG	CAGCATGATC	GAAACATACA	GTCAACCTTC	TCCCCGCTCC	GTGGCCACTG	60
GACCACCTGT	CAGTATGAAA	ATTTTTATGT	ATTTACTTAC	AGTTTTTCTT	ATCACCCAGA	120
TGATTGGGTC	AGCGCTTTTT	GCTGTGTATC	TTCACAGACG	ATTGGACAAG	ATAGAAGACG	180
AAAGGAATCT	TCATGAAGAT	TTTGTGTTCA	TGAAAACGAT	ACAGAGATGC	AATAAAGGAG	240
AGGGGTCCTT	ATCCTTACTG	AACTGTGAGG	AAATTAGAAG	CCGGTTTGAA	GACTTGGTCA	300
AGGATATAAT	GCAAAACAAA	GAAGTAAAGA	AGAAAGAAAA	AAACTTTGAA	ATGCACAAGG	360
GTGATCAGGA	GCCTCAGATA	GCGGCACATG	TCATCAGTGA	GGCCAGTAGT	AAAACAACCT	420
CTGTTCTCCA	GTGGGCCCCC	AAAGGATACT	ACACCCTAAG	CAACAACCTG	GTAACCCTCG	480
AAAACGGGAA	ACAGCTGGCC	GTGAAAAGAC	AAGGATTCTA	TTACATCTAC	ACCCAAGTCA	540
CCTTCTGTTT	CAATCGGGAA	ACTTTGAGTC	AAGCTCCATT	TATAGCCAGC	CTCTGCCTGA	600
AGTCCCCAAG	TGGATCAGAG	AGAATCTTAC	TGAGAGCTGC	AAACACCCAC	AGTTCTTCCA	660
AACCATGCGG	GCAGCAATCC	ATTCACCTAG	GAGGAGTCTT	TGAATTGCAA	TCGGGTGCTT	720
CGGTGTTTGT	CAATGTGACT	GATCCAAGTC	AAGTGAGCCA	CGGGACGGGC	TTCACATCAT	780
TTGGCTTACT	CAAACTCTGA	ACGGTGTAAG	CCAGCAGGCT	GCGGCTGGGC	TGATGCTGGT	840
GGTCTTCACA	ATCCAGGAAA	GCAG				864

## (2) INFORMATION FOR SEQ ID NO: 9:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3634 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GAATTCCGGG	TGATTTCACT	CCCGGCTGTC	CAGGCTTGTC	CTGCTACCCC	ACCCAGCCTT	60
TCCTGAGGCC	TCAAGCCTGC	CACCAAGCCC	CCAGTCTCCT	CTCCCCGCAG	GACCCAAACA	120
CAGGCCTCAG	GACTCAACAC	AGCTTTTCCC	TCCAACCCGT	TTTCTCTCCC	TCAACGGACT	180
CAGCTTTCTG	AAGCCCCCTC	CAGTTCTAGT	TCTATCTTTT	TCCTGCATCC	TGTCTGGAAG	240
TTAGAAGGAA	ACAGACCACA	GACCTGGTCC	CCAAAAGAAA	TGGAGGCAAT	AGGTTTTGAG	300
GGGCATGGGG	ACGGGGTTCA	GCCTCCAGGG	TCCTACACAC	AAATCAGTCA	GTGGCCCGAG	360
AGACCCCTCT	CGGAATCGGA	GCAGGGAGGA	TGGGGAGTGT	GAGGGGTATC	CTTGATGCTT	420
GTGTGTCCCC	AACTTTCCAA	ATCCCCGCCC	CCGCGATGGA	GAAGAAACCG	AGACAGAAGG	480
TGCAGGGCCC	ACTACCGCTT	CCTCCAGATG	AGCTCATGGG	TTTCTCCACC	AAGGAAGTTT	540
TCCGCTGGTT	GAATGATTCT	TTCCCCGCCC	TCCTCTCGCC	CCAGGGACAT	ATAAAGGCAG	600
TTGTTGGCAC	ACCCAGCCAG	CAGACGCTCC	CTCAGCAAGG	ACAGCAGAGG	ACCAGCTAAG	660
AGGGAGAGAA	GCAACTACAG	ACCCCCCTTG	AAAACAACCC	TCAGACGCCA	CATCCCCCTGA	720
CAAGCTGCCA	GGCAGGTTCT	CTTCCTCTCA	CATACTGACC	CACGGCTTCA	CCCTCTCTCC	780
CCTGGAAAGG	ACACCATGAG	CACTGAAAGC	ATGATCCGGG	ACGTGGAGCT	GGCCGAGGAG	840
GCGCTCCCCA	AGAAGACAGG	GGGGCCCCAG	GGCTCCAGGC	GGTGCTTGTT	CCTCAGCCTC	900
TTCTCCTTCC	TGATCGTGCC	AGGCGCCACC	ACGCTCTTCT	GCCTGCTGCA	CTTTGGAGTG	960
ATCGGCCCCC	AGAGGGAAGA	GGTGAGTGCC	TGGCCAGCCT	TCATCCACTC	TCCCACCCAA	1020
GGGGAAATGA	GAGACGCAAG	AGAGGGAGAG	AGATGGGATG	GGTGAAAGAT	GTGCGCTGAT	1080
AGGGAGGGAT	GAGAGAGAAA	AAAACATGGA	GAAAGACGGG	GATGCAGAAA	GAGATGTGGC	1140
AAGAGATGGG	GAAGAGAGAG	AGAGAAAGAT	GGAGAGACAG	GATGTCTGGC	ACATGGAAGG	1200
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AATAAGATAT	GGAGACAGAT	GTGGGGTGTG	AGAAGAGAGA	TGGGGGAAGA	AACAAGTGAT	1320
ATGAATAAAG	ATGGTGAGAC	AGAAAGAGCG	GGAAATATGA	CAGCTAAGGA	GAGAGATGGG	1380
GGAGATAAGG	AGAGAAGAAG	ATAGGGTGTC	TGGCACACAG	AAGACACTCA	GGGAAAGAGC	1440
TGTTGAATGC	TGGAAGGTGA	ATACACAGAT	GAATGGAGAG	AGAAAACCAG	ACACCTCAGG	1500
GCTAAGAGCG	CAGGCCAGAC	AGGCAGCCAG	CTGTTCCTCC	TTTAAGGGTG	ACTCCCTCGA	1560
TGTTAACCAT	TCTCCTTCTC	CCCAACAGTT	CCCCAGGGAC	CTCTCTCTAA	TCAGCCCTCT	1620
GGCCCAGGCA	GTCAGTAAGT	GTCTCCAAAC	CTCTTTCCTA	ATTCTGGGTT	TGGGTTTGGG	1680
GGTAGGGTTA	GTACCGGTAT	GGAAGCAGTG	GGGGAAATTT	AAAGTTTTTG	TCTTGGGGGA	1740
GGATGGATGG	AGGTGAAAGT	AGGGGGGTAT	TTTCTAGGAA	GTTTAAGGGT	CTCAGCTTTT	1800
TCTTTTCTCT	CTCCTCTTCA	GGATCATCTT	CTCGAACCCC	GAGTGACAAG	CCTGTAGCCC	1860
ATGTTGTAGG	TAAGAGCTCT	GAGGATGTGT	CTTGGAACCT	GGAGGGCTAG	GATTTGGGGA	1920
TTGAAGCCCG	GCTGATGGTA	GGCAGAACTT	GGAGACAATG	TGAGAAGGAC	TCGCTGAGCT	1980
CAAGGGAAGG	GTGGAGGAAC	AGCACAGGCC	TTAGTGGGAT	ACTCAGAACG	TCATGGCCAG	2040
GTGGGATGTG	GGATGACAGA	CAGAGAGGAC	AGGAACCGGA	TGTGGGGTGG	GCAGAGCTCG	2100
AGGGCCAGGA	TGTGGAGAGT	GAACCGACAT	GGCCACACTG	ACTCTCCTCT	CCCTCTCTCC	2160
CTCCCTCCAG	CAAACCTCA	AGCTGAGGGG	CAGCTCCAGT	GGCTGAACCG	CCGGGCCAAT	2220
GCCCTCCTGG	CCAATGGCGT	GGAGCTGAGA	GATAACCAGC	TGGTGGTGCC	ATCAGAGGGC	2280

CTGTACCTCA TCTACTCCCA GGTCCCTCTTC AAGGGCCAAG GCTGCCCCCTC CACCCATGTG 2340  
 CTCCTCACCC ACACCATCAG CCGCATCGCC GTCTCCTACC AGACCAAGGT CAACCTCCTC 2400  
 TCTGCCATCA AGAGCCCCCTG CCAGAGGGAG ACCCCAGAGG GGGCTGAGGC CAAGCCCTGG 2460  
 TATGAGCCCA TCTATCTGGG AGGGGTCTTC CAGCTGGAGA AGGGTGACCG ACTCAGCGCT 2520  
 GAGATCAATC GGCCCGACTA TCTCGACTTT GCCGAGTCTG GGCAGGTCTA CTTTGGGATC 2580  
 ATTGCCCTGT GAGGAGGACG AACATCCAAC CTTCCCAAAC GCCTCCCCTG CCCCATCCC 2640  
 TTTATTACCC CCTCCTTCAG ACACCCTCAA CCTCTTCTGG CTCAAAAAGA GAATTGGGGG 2700  
 CTTAGGGTCG GAACCCAAGC TTAGAACTTT AAGCAACAAG ACCACCACTT CGAAACCTGG 2760  
 GATTGAGGAA TGTGTGGCCT GCACAGTGAA GTGCTGGCAA CCACTAAGAA TTCAAACTGG 2820  
 GGCCTCCAGA ACTCACTGGG GCCTACAGCT TTGATCCCTG ACATCTGGAA TCTGGAGACC 2880  
 AGGGAGCCTT TGGTTCTGGC CAGAATGCTG CAGGACTTGA GAAGACCTCA CCTAGAAATT 2940  
 GACACAAGTG GACCTTAGGC CTTCTCTCTT CCAGATGTTT CCAGACTTCC TTGAGACACG 3000  
 GAGCCCAGCC CTCCCCATGG AGCCAGCTCC CTCTATTTAT GTTTGCACTT GTGATTATTT 3060  
 ATTATTTATT TATTATTTAT TTATTTACAG ATGAATGTAT TTATTTGGGA GACCGGGGTA 3120  
 TCCTGGGGGA CCAATGTAG GAGCTGCCTT GGCTCAGACA TGTTTTCCGT GAAAACGGAG 3180  
 CTGAACAATA GGCTGTTCCC ATGTAGCCCC CTGGCCTCTG TGCCTTCTTT TGATTATGTT 3240  
 TTTTAAAATA TTTATCTGAT TAAGTTGTCT AAACAATGCT GATTGTGTGA CCAACTGTCA 3300  
 CTCATTGCTG AGCCTCTGCT CCCCAGGGGA GTTGTGTCTG TAATCGCCCT ACTATTCAGT 3360  
 GGCGAGAAAT AAAGTTTGCT TAGAAAAGAA ACATGGTCTC CTTCTTGGA TTAATTCTGC 3420  
 ATCTGCCTCT TCTTGTGGGT GGGAAGAAGC TCCCTAAGTC CTCTCTCCAC AGGCTTTAAG 3480  
 ATCCCTCGGA CCCAGTCCCA TCCTTAGACT CCTAGGGCCC TGGAGACCCT ACATAAACAA 3540  
 AGCCCAACAG AATATTCCCC ATCCCCCAGG AAACAAGAGC CTGAACCTAA TTACCTCTCC 3600  
 CTCAGGGCAT GGAATTTCC AACTCTGGGA ATTC 3634

## (2) INFORMATION FOR SEQ ID NO: 10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1997 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GAGACAGAGT CTGCTCTGT CCCCAGGCT GGAATACAGT GGTGCGATCT TGACTCACTG 60  
 CAGCCTCCGC CTCCCAGGTT CAAATAATTC TCCAGCCTCA GCCTCCCGAG TAGCTGGGAC 120  
 TGCAGATGCG CACCAGCACG CCTGGCTAAT TTTTGTATTT ATTATAGAGA TGGGGTTTCA 180  
 CCATGTTGGC CAGCTGGTCT CAAACTCCTG ACCTCAAGTA ATCCGCCCCAC CTCAGACTCC 240  
 CAAAGTGCCA GGATTACAGG TGTGAGCCAC TGCACCAGGC CTGGAACAAT TTTAAAATAA 300  
 TGTATTGGCT CTGCAATGTC AGCTTCAGAA CAAGTCCCTT AGCTGTCCCC ACCCCACCCT 360  
 AAGTCACCAC CCTTAAGCCT CACCCATGTG GAATTCTGAA ACTTCCTTTG TAGAAAACCT 420  
 TGGAAGGTGT CTGCCACATT GATCCTGGAA TGTGTGTTTA TTTGGGGTTA TATAAATCTG 480  
 TTCTGTGGAA GCCACCTGAA GTCAGGAAGA GATGGAGGGC ATCCTTCAGG AGTGAGATGA 540  
 GACCTCATCA TACTTGACTG TCCAGCATCA TCTCTGAGTA AGGGGACCAA AAAATTTATC 600  
 TTCCAAACTA GGACACTTTC AAGAGTGGA GGGGGATCCA TTAATATTTT CACCTGGACA 660

AGAGGCAAAC	ACCAGAATGT	CCCCGATGAA	GGGGATATAT	AATGGACCTT	CTTGATGTGA	720
AACCTGCCAG	ATGGGCTGGA	AAGTCCGTAT	ACTGGGACAA	GTATGATTTG	AGTTGTTTGG	780
GACAAGGACA	GGGGTACAAG	AGAAGGAAAT	GGGCAAAGAG	AGAAGCCTGT	ACTCAGCCAA	840
GGGTGCAGAG	ATGTTATATA	TGATTGCTCT	TCAGGGAACC	GGGCCTCCAG	CTCACACCCC	900
AGCTGCTCAA	CCACCTCCTC	TCTGAATTGA	CTGTCCCTTC	TTTGGAAGTC	TAGGCCTGAC	960
CCCCTCCCT	GGCCCTCCCA	GCCCACGATT	CCCCTGACCC	GACTCCCTTT	CCCAGAACTC	1020
AGTCGCCCTGA	ACCCCCAGCC	TGTGGTTCTC	TCCTAGGCCT	CAGCCTTTCC	TGCCTTTGAC	1080
TGAAACAGCA	GTATCTTCTA	AGCCCTGGGG	GCTTCCCCCG	GCCCCAGCCC	CGACCTAGAA	1140
CCCGCCCGCT	GCCTGCCACG	CTGCCACTGC	CGCTTCCCTCT	ATAAAGGGAC	CTGAGCGTCC	1200
GGGCCCAGGG	GCTCCGCACA	GCAGGTGAGG	CTCTCCTGCC	CCATCTCCTT	GGGCTGCCCG	1260
TGCTTCGTGC	TTTGGAATAC	CGCCAGCAG	TGTCCTGCCC	TCTGCCTGGG	CCTCGGTCCC	1320
TCCTGCAGCT	GCTGCCTGGA	TCCCCGGCCT	GCCTGGGCCT	GGGCTTGGTG	GGTTTGGTTT	1380
TGGTTTCCCT	CTCTGTCTCT	GACTCTCCAT	CTGTCACTCT	CATTGTCTCT	GTCACACATT	1440
CTCTGTTTCT	GCCATGATTC	CTCTCTGTTT	CCTTCCTGTC	TCTCTCTGTC	TCCCTCTGCT	1500
CACCTTGGGG	TTTCTCTGAC	TGCATCTTGT	CCCCTTCTCT	GTCGATCTCT	CTCTCGGGGG	1560
TCGGGGGGTG	CTCTCTCCCA	GGGCGGGAGG	TCTGTCTTCC	GCCGCGTGCC	CCGCCCCGCT	1620
CACTGTCTCT	CTCTCTCTCT	CTCTTTCTCT	GCAGGTTCTC	CCCATGACAC	CACCTGAACG	1680
TCTCTTCTCT	CCAAGGGTGT	GTGGCACCAC	CCTACACCTC	CTCCTTCTGG	GGCTGCTGCT	1740
GGTTCTGCTG	CCTGGGGCCC	AGGTGAGGCA	GCAGGAGAAT	GGGGGCTGCT	GGGGTGGCTC	1800
AGCCAAACCT	TGAGCCCTAG	AGCCCCCTC	AACTCTGTTT	TCCCCTAGGG	GCTCCCTGGT	1860
GTTGGCCTCA	CACCTTCAGC	TGCCCAGACT	GCCCGTCAGC	ACCCCAAGAT	GCATCTTGCC	1920
CACAGCACCC	TCAAACCTGC	TGCTCACCTC	ATTGGTAAAC	ATCCACCTGA	CCTCCAGAC	1980
ATGTCCCCAC	CAGCTCT					1997

## (2) INFORMATION FOR SEQ ID NO: 11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10240 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GAATTCCTCCG	GATCAAAGTC	AGCATTAAAT	CCCAGTTTAT	GTTTTGAGGC	TAAGTTCAAG	60
TTTGAGTCTA	ATGTCATTTT	AGCCTTGTTT	GGAGGACTCA	GAGATTTTAC	TAGTTTCTCC	120
GCAGAGACCA	CTGTAGAAAC	TGCATTTCCC	TGAGTTTTGG	GCACAAGACT	CCAGTCATCA	180
CCCCTCCCAC	ACAGGGAAAG	CCCCAAACCA	ACTGCTGGCC	TCCTCAAGAA	AGAAACCGAA	240
TTTCACACAA	CCTCCGAAAC	TAAGATTGAA	ACCAAGATTG	GCCCATCTCA	AGGCGCGTCC	300
TCCAGCACAT	TGAGAAATGC	GCTGATGGAG	CCTCGGCCCA	GCTCTCGAGC	TTCTTCTCCT	360
TCTGTCTCTC	ATGTCTTCTC	ATCACTCCTT	CTCACCTTCC	CGTTTTTGTG	CTGCAATGCC	420
CCCTTCTTCC	TCTCTTCTCT	GGGTTTTTCC	CTTTATTTCT	CACTGTACCA	TTTTATATTT	480
TAATAAAGCC	GAGGTCTCCT	AGTCCATCAG	CTCCTACTGT	TGGAGAGGAG	GCAGAAAGAA	540
ACAGCAGGAC	GGCAAAGGGA	CTCCAGAGAA	AGAGACTCAG	AGGAAAGGCA	AGAAACAGGG	600
ACCAAGAGAG	AGGCCAACAG	TGACACAAGA	CACAGTGAGG	TTAAAAGAAA	TAAGATGAGG	660
CCAAGATAGA	GACCAAGCTA	TTTAAAAGAG	CCATCTGTGG	CTACCCCTTCT	TCCGCCATCG	720
CATCTGGTCA	GCCACCAAGA	TTTTGCCTAG	AAACGTTTCT	CCTCTCCATT	CTCCTGCTGC	780



TGCTGCTGCT	GCTGCTGCTG	CTGCTGCTGC	TGCTGCTGCT	GCTGCTGCTG	CTGCCTTAAT	840
ACGAATGCAG	GCTCTTGTCA	TCTCCTTGCT	GGGTGTGTC	AAAATCCTCC	TAAGTGGTCT	900
CCACACTTCT	CATTTCCCTT	CCAGCCCCCT	ATCTTCCATA	CTTCCATTTA	TTTATTTTGG	960
CCATGCCCAT	GGCATGTGGC	AGTTCCAGGG	GCCAGGGATC	AAACCTGTGC	CAATGCAGTG	1020
ACCGTGTGAG	ATCCTTAACC	CACTGCACAC	AAGGCAACGC	CCCTCGAGTC	ATTCTCATTT	1080
TTTAAATATA	CCAATTTGAG	GGGGTCCCTC	TTTCACTTAA	AAATTTTGGC	AGTCCCTAT	1140
CATGATGAGA	AGGAATTCCA	AACCATTTTT	CTTGTGTGCA	AACCCCTCAG	CATGTGTCTT	1200
CAGCTTACTT	CCCAAGCCTC	ATCCCTGCTC	CTTCTACGTG	TACCCATGTG	TACATCTCCA	1260
CACACCATAT	ACTCTTTTTT	ACCTCCCATC	TTTGCACCTT	CTGTTCCCTC	TCTCTGCCCC	1320
TCACCATCTT	TTTTGCTTTG	ATACTTAATG	CCTCTCCCTC	AGGCCAGGTT	CAATGGCTTT	1380
TCTGTGGGCT	GCTTTAAGCC	CACTGTCTAT	GAACCTATCA	CATTTTATTT	TATTTGACTT	1440
TCTTTTTTAG	CCCGCACCCA	GCATATGGAG	ATTTCCAGGC	TAGGGATCTA	ATCGGAGCTG	1500
TATCTGCCAG	CCTGCGCTGG	AGCCACAGCA	ACGTGGGATC	CGAGCCTGAG	GGGTTTTGAT	1560
GTCTGTGGC	ACAGAAGTTA	CATTCAGGCT	GTGCATGAAC	TATTTCTCCT	GTTCTCCTCC	1620
CCCTGCTTGA	GGCCCTGCAG	CTTTGCCTCT	CATGCCTTGC	TGCTCTGACC	TATGACTTCT	1680
TTTTGTTTGC	ATTCCATCTC	TTTAGTTTTT	TCTCTGTTCC	ACAAACATTT	ACTGAGCATC	1740
TACATGAGGC	ATTGAGGATA	CGGATGGGAA	AGACAGTCCC	CTGACCTCTG	GGACCTCAAA	1800
GACCAATTGT	GGAAGACTGG	TTGGTTATCA	GATAATTACA	ATGAAGTGTG	GGAGTCCCTG	1860
TCATGGGTCA	GCAGGTAATG	AACCCAGTAA	ACGATCCATG	AGGATGCAGA	TTCAATCCCT	1920
GGCCTTGCTC	AGCGGGTTAA	GGATCCAGCG	TTCCACAAAG	CTGTGGTGTA	GGTCGCAGAT	1980
GCGACTCAGA	TCTTGCAATT	CTGTGGCTGT	GGTGTAGGCT	GGTGGCTACC	CCTAGCCTGG	2040
GAACCTCCAT	ATGCCTCAGG	TGCGGCCCTA	AAAGACAAAA	AAAAAAAAGA	GAGAACTTTT	2100
TCTTTTTCTT	AATGTGTAAC	CTACAAGCTA	AGTGAAGAACT	GGCTCCTATT	CCATAACGTT	2160
TGTATCATTT	TTCATACTAG	CCAAATACTA	GAAACAGGGA	GTTCCCGTCG	TGGTGCAGCA	2220
GAAACAAATT	CGACTAGGAA	CCATGAGGTT	GCGGGTTCGA	TCCCTGGCCT	TGCTCAGTGG	2280
GTTAAGGATC	CGGCGTTGCC	GTGAGCTGTG	GTGTAGGTCG	CAGATGTGGC	TCGGATCTAG	2340
TGTTGCTGTG	GCTCTGGTGT	AGGCCGGCAG	CAACAGCTCT	GATTAGACTC	CTAGCCTGAG	2400
AACCTCCATA	AGCTGTGGCT	GCGGCCCTAT	AAAGACAAAA	AAAAAAAAAA	GGCCAAATAC	2460
TAGAAAACAA	CCAAATGCCC	ATCAACAGAA	GAATAGATAA	GTTAATTGGG	GTATATGCAC	2520
ACAATAGCAT	CACACAATAA	CATGCACACA	ATAACATCAC	AATGAAATAA	AAATTACTAC	2580
TGACAGACAC	AACCATATAG	ATGAATTTCA	CAAACACAAC	AGCGAGAATA	AAAGCCAAGC	2640
ACAGATGAGT	TGCTGTGTGT	GATTTCATTT	TATGAAGTTC	AAGCGCAGGA	AGAACTTAAT	2700
CTATAGTGAC	AGAGGTCAGA	GAGCAGTTGG	TTGTCTTTGG	CAGGTATGAA	CTGGGAGTGG	2760
GCATGAGAGA	ACTTTCCTGA	GACCTAAAAA	TATATTGGAC	TGGATGGTGG	CAACATGGCT	2820
ACAAGAAGAT	GGAAAAGTTC	CTCAGGCTGT	CCACTTGGGA	GACGGGCTTC	TCACGGGACC	2880
TAAGTTCTGC	ATCAGCAGAG	GGGGAATATC	TTAATGATTT	GACAATTACA	AAGTGTATTG	2940
GCTTTACCGA	TGTATTTTCA	ACACAATCCC	TCTGCTGTCC	CCACCCACC	CTAGGTCACC	3000
ACCCTTAAGC	TCCACCTGTG	TGGAATTCTG	AAGCCTCCCC	TGTAGAGAAC	TTTAGCAGTT	3060
GCCACGTTCT	TTTGATGCAG	GAACGTGTTG	TCTAGAGTTA	GACACATCTG	ATCTGTGGGG	3120
CCCACCCAAG	GTTGGGACAT	GGTGGGGGGC	GGCCTTCTGC	AGTGAGATGA	AACCTCATTT	3180
TAGGTGATTT	CGTGGCCTCA	TCCCTGAGTC	AGATCTTCCA	AATGAGGACA	CTTTGGAGAG	3240
CAAAAGGGGG	CTCCCTGAAG	ATTTCTCTCA	GGACAGCAGG	AACAAACCAG	GATGTCCAG	3300
GCAGGAGGGT	ATAGAAGGGA	ACTTGTTGAT	ATGAAATCAG	CCAGATGACC	TGGAAAATAC	3360
ACAGACTGGG	ACAAGTGTGA	CTTGAGCCTC	TTGGGCCCCAG	GACAGGGGTA	CAGAGGAGGA	3420
AACGTGCACA	GAGAGAAGCC	CGTAATCAGC	CAAGGCTGCA	GAGGTGTTAT	ACATAATCGC	3480
TCTTCACGCA	ACCGGGCAAG	CAGCCCACGC	CCCAGCTGCA	CTCCATCTCC	TCCTCTGAAC	3540
TCACCGTCCC	TTCTCTGGAA	CTCCTAAGCC	TGACCCCGCT	CCCTGGCCCT	CCCAGCCAC	3600
GGTTCCCTTG	ACCCCACTCC	CTTTCCCGAG	ACTCAGTCAT	CTGAGCCCCC	AGCCTGCGTT	3660
CTCTCCTAGG	CCTCAGCCTT	TCCTGCCTTC	GCGTGAAACA	GCAGCATCTT	CTAAGCCCTG	3720

GGCTTCCCCA	GGCCCCAGCC	CCGGCCTAGA	ACCCGCCCAG	CCGACCTGCC	CACGCTGCCA	3780
CTGCCGGCTT	CCTCTATAAA	GGGACCCAGG	GCGCCCAGAA	AGGGGCCCAC	AGGGGTCCCG	3840
CACAGCAGGT	GAGACTCTCC	CACCCCATCT	CCTAGGGCTG	TCCGGGTGCT	GGACTCCCCC	3900
CTCACTTCGG	TCCCTCCGCC	CGCTCCCTGG	CCTTCCTGCC	CCTCCTGCAT	CTTCACCCCG	3960
GCCTGGGCCCT	TGGTGGGTTT	GGTTTTGGTT	TGTTCTCTCT	GATTCTTTAT	CTGTCAGGCT	4020
CTTTCTAGCT	CTCACACACT	CTGATCCCTC	TCTGTTCCCT	TCCCATCTCT	GTTTCTCTCT	4080
GGGTCTCCCC	CTGCTCACCT	CGGGATTTC	CTGAGTGCCT	CTGGTCCCCCT	TCTCTGTCTG	4140
GCGCCCCGTC	TCTTGCTCT	CGGGGTGGCT	GTCTCCGAGG	GCAGGAGGCC	TTCTTCCGCA	4200
GGTGCCCCGC	CCCGCTCACT	GTCTCTCTCC	CCCCACAGGT	TTTCCCCATG	ACACCACCTG	4260
GACGCCTCTA	CCTCCGGAGG	GTGTGCAGCA	CCCCATCCT	CCTCCTCCTG	GGGCTGCTGC	4320
TGGCCCTGCC	GCCCCAGGCC	CAGGTGAGCA	AGCAGAGAG	CGGGCCGTGG	TGGCAGCCCT	4380
CGCCAACTTT	GGGCCTCAGA	GCCTCTCTGA	CGCTCTTCTC	CCCTAGGGGC	TCCCTAGGCGT	4440
CGGCCTCCCA	CCCTCAGCTG	CACAGCCTGC	CCATCAGCAC	CCCCAAAGC	ACTTGGCCAG	4500
AGGCACCCTC	AAACCTGCCG	CTCACCTCGT	TGGTAAACAT	CCACCTGGCC	TCCCAGACCT	4560
GTAGCCCCCA	GTCTCTCTCC	TATGCCCCCTG	CTTCAGGGAC	TGAAGCATCC	CTCCCCCCCCA	4620
TCTCCCCCCA	CCCCCTAAAT	GGAGGCATCC	CACTCCCGAC	TCCCTCCCCAA	CCATCCCCCA	4680
GGAAGTCAGT	CCAGCACCTG	CTTCCTCAGG	GATTGAGACC	TCCGACCCCC	AGGTCCTTGA	4740
CTCCCACCCC	CTCTGGCTCT	TCCTAGGAGA	CCCCAGCACC	CCGGACTCAC	TGCGCTGGAG	4800
AGCGAACACG	GATCGTGCCT	TCCTCCGCCA	TGGCTTCTTG	CTGAGCAACA	ACTCCCTGCT	4860
GGTCCCCACC	AGTGGCCTCT	ACTTTGTCTA	CTCCCAGGTC	GTCTTCTCCG	GGGAAGGCTG	4920
CTTCCCCAAG	GCCACCCCCA	CCCCTCTCTA	CCTGGCCAC	GAGGTCCAGC	TCTTCTCCTC	4980
CCAGTACCCC	TTCCACGTGC	CGCTCCTCAG	CGCTCAGAAG	TCCGTGTGCC	CCGGGCCACA	5040
GGGACCTTGG	GTGCGCTCTG	TGTACCAGGG	GGCTGTGTTT	CTGCTCACCC	AGGGAGATCA	5100
GCTGTCCACA	CACACAGACG	GCACCCCCCA	CCTGCTCCTC	AGCCCCAGTA	GCGTCTTCTT	5160
TGGAGCCTTC	GCTCTATAGA	AGAATCCAGA	AAGAAAAAAA	TTGGTTTCAA	GGCCTTCTCC	5220
CCTTTTCACC	TCCCTTATGA	CCACTTCGGA	GGTCACCGCG	CCTCTCCTCT	GACAATTTCC	5280
AACAGTCTCA	TCTTCCCCCA	CGCTCAGCAC	CTGGAGCTTC	TGTAGAAGGA	ATTCTAGGCA	5340
CCTCGGGGGA	ACTGGAACCA	CCCCGGATGC	TCTGCTGAGG	ATCTGAATGC	CCGCCTGGAG	5400
CCCTTCCCCCT	GTCCTGCCCG	TCTAGGGGCC	CTCGTCCAGG	ACGTGGAAGG	GAAGCTGACC	5460
CATGAGGGAC	TTTGAACGGA	TGACCGGAGC	GGTGTGGGGG	GGTTATTTAT	GAAGGGGAAA	5520
ATTAAATTAT	TTATTTATGG	AGGATGGAGA	GAAGGGAATC	ACAGAGGGAT	GTCAGAAGAG	5580
TGTGACACAT	GTGCCCAAGA	GATAAAGTGA	CAGAAGGCAT	GGGCTCCAGA	TGACCCGGCC	5640
AGAGAGGGCA	AAGTGGCTCA	GGAAGGGGCT	GCTTGACTGG	AGGCTCATGA	GGAGACGGCT	5700
GACCCTCGAT	GAAACCCAAT	AAAGCTCTTT	TCTCTGAAAT	GCTGTCTGCT	CGTATCTGTC	5760
ACTCGGGAGG	GGAGAATTCT	CCAGATGTCT	CTAAGGAGTG	GAGGGAGGAC	AGGAATCAGA	5820
GGGGACGGGA	GCTGTGGGTG	TGTGATGAGG	CCTAAGGGGC	TCAGGTGAGA	GATGGCGGCC	5880
TCAGGGTGAG	GGCAGCCAGA	CCCCTGCAGG	AGAAGCAGAT	GGTTCTCTG	AGAAGACAAA	5940
GGAAGAGATG	CAGGGCCAAG	GTCTTGAGAA	CCGAGGTCGG	GGGTCGCCCTG	GCAGATATGG	6000
CCACAGGTAG	AGGGACAGAG	GAATAGGGGT	GACAGGAGGC	TTCCCGGGAG	AAGGGAACAC	6060
ACTGAGGGGT	GTTCCGGGAT	CTGAGGGAGG	AGCACGGGGA	CGCCCTGGGA	GACATGCCGT	6120
CCAGGGCCAT	GAGGAGTGGG	AGAGCCTCTG	AGGCTAGCGG	CTGGAGATAC	AGGGACATTT	6180
GAGGAGACAC	GGTCATGGCC	AGGAGCCGCG	AGGGCCTGGA	CAGTCTCTAG	GAATCTCGAA	6240
GAAGCAGGAA	TTCTTTGAGG	ATACGTGGCC	ACACAAAGGG	AGGCTGAGGT	GTGGGGACTT	6300
CATGCAGAAG	TCAGGGCCTC	ACATTCCCTT	GGAAGCCGAG	ACTGAAACCA	GCAGCAGAGT	6360
TTTGGTGAGT	TCCTGTGAGA	GTGAAAGGAG	AAGGCCCGCC	ATGGTGGGTT	TGTGAATTCC	6420
CAGCCTGGCT	TCCTCTCCCT	CTGGGGCTGT	CCCAGGCCTG	TTCTTGCCGT	CCTCCCCCAG	6480
CCCGTGTAGG	GCCTCCAGCT	GCCCTTCTCC	CAGCTCCTCT	TCCCTCCAGG	AGACGAAACA	6540
TGGGTCTCAG	CACCCAGCGC	GGTGTGCTCT	AAGTTTTCTC	TCCATTAAGA	ACTCAGCTTT	6600
CTGAAGCTCC	TCCCATTCTT	AGTTCTACCC	CTACCTGAGC	CCTGTTCCGA	AATCAGAGAG	6660

AAATAGAAAGT	CATCCCCCAA	AGAAAAGGAA	TTTGTCCCCC	AAAGAAACAG	AACTTGTCCC	6720
CCAAAGAAAT	GGAAACAATG	GGAAATGGGA	GGCAGGGGGG	ACCTGGGGTC	CAGCCTCCAG	6780
GGTCTACAC	ACAGAGCAGT	AACTGGCCCA	GCAAGCCCAC	CTCAGGATCC	GGGCAGGGAG	6840
GGTAGGAAGT	ATCCCTGATG	CCTGGGTGTC	CCCAACTTTC	CAAACCGCCG	CCCCCGCTAT	6900
GGAGATGAAA	CTAAGACAGA	AGGTGCAGGG	CCCGCTACCG	CTTCCTCCAG	ATGAGCTCAT	6960
GGGTTTCTCC	ACCAAGGAAG	TTTTCCGCTG	GTTGAAAGAG	AGCCTCTCCC	CGCCCTCTTC	7020
TCACCCAGAG	CGTATAAATG	CAGCTGTTTG	CACACCCAGC	CAGCAGAAGC	TCCCAGAGTG	7080
AGGACACCAG	GGGACCAGCC	AGGAGAGAGA	CAAGCCATCT	CCAGGACCCC	CTAGAAATAA	7140
CCTCTCAGAA	GACACACCCC	CGAACAGGCA	GCCGGACGAC	TCTCTCCCTC	TCACACGCTG	7200
CCCCGGGGCG	CCACCATCTC	CCAGCTGGAC	CTGAGCCCCC	CTGAAAAAGA	CACCATGAGC	7260
ACTGAGAGCA	TGATCCGAGA	CGTGGAGCTG	GCGGAGGAGG	CGCTCGCCAA	GAAGGCCGGG	7320
GGCCCCCAGG	GCTCCAGGAG	GTGCCTGTGC	CTCAGCCTCT	TCTCCTTCCT	CCTGGTCGCA	7380
GGAGCCACCA	CGCTCTTCTG	CCTACTGCAC	TTCGAGGTTA	TCGGCCCCCA	GAAGGAAGAG	7440
GTGAGCGCCT	GGCCAGCCTT	GGCTCATTCT	CCCACCCGGA	GAGAAATGGG	GAAGAAAGAG	7500
GGCCAGAGAC	GAGCTGGGGG	AAAGAAAGTGT	GCTGATGGGG	AGTGTGGGGA	GGAAATCATG	7560
GAGAAAGATG	GGGAGGCAGA	AGGAGACGTG	GAGAGAGATG	GGGGGAGAGA	GAGAAGGATG	7620
GAGAGAAATC	CGGTGGCCCC	GCCCTTGGA	ATGCTCTCTA	AATATTTGTT	GCACGAATGA	7680
GTGAGTAAGC	AGGGACACCG	ATATAAAGAG	AGATGAGTAG	ACAGACAAGG	GGTGTGGTAG	7740
AAAGATAGGG	AAAAAACAAG	TGATCTGGAT	AAAGATAGTG	AGACAGGAAG	AGGTAGAGGA	7800
GATAGGAAAG	AGAGATAAGG	AGAGAAGAAG	GAAGCGTGGG	TGTCTGGCAC	GTGGAAGGCA	7860
CTCAATGAAG	GAGTTGTTGA	ATGGATGGGT	GGATGAGAAA	ATGGATGAGT	GGAGAGAAAA	7920
AACTAGACAT	CAGGGCAGAG	AGTACAAGCT	AGAGAAGCAG	GTGGCTGTTT	TCCCTTCAGA	7980
GGGGACTTAT	TCAAATCTAA	TTAATCCTTC	TTCTTCTCCC	CAACAGTTTC	CAGCTGGCCC	8040
CTTGAGCATC	AACCCTCTGG	CCCAAGGACT	CAGTAAGTAT	CTCTAAACC	TGTCTCTCAG	8100
TTCTGAGCTT	GGACAGGGGT	GGGGTATAGT	CTGGGGTGGA	AGGAAGAAAG	GAAATTTAGG	8160
GTCTGGGTTT	GGCGGGGGGA	ATGCAGGTCA	AAGTAGTGAG	ATATTTTCTG	GGAAGTCTGA	8220
GGGTCTCATC	TTTTTCTTTC	CTCTTCTCTC	CTCAGGATCA	TCGTCTCAAA	CCTCAGATAA	8280
CCCCGTCCGC	CACGTTGTAG	GTAAGAGTTC	TGAGGATGTG	TCTGGGGGAT	GAAGAAATAG	8340
GCAGGACAGA	GAGGTATAGG	ATTTGGGGGC	TGAAGCCAGG	CTGAGGGTAG	CCAGAGCTTG	8400
GAGATAGTAT	GAGGAGGACT	CGCTGAGCTC	CAGGGGAGGA	TGGGGGATAC	TCAGAACTTG	8460
AGGAGGATAC	TCGGAACCTC	ATGGACAGAT	GGGATGTGGG	AAGACAGACC	GAGGGGACAG	8520
GAACCGGATG	TGGGGGGCGG	GCAGAACTCG	AGGGCCAGGA	TGTGGAGAGT	GGAACTGACA	8580
GGGTCACACT	GACTCACCCC	TCCCTCTTTG	TCTCCTCCCT	CCAGCCAATG	TCAAAGCCGA	8640
GGGACAGCTC	CAATGGCAGA	GTGGGTATGC	CAATGCCCTC	CTGGCCAACG	GCGTGAAGCT	8700
GAAAGACAAC	CAGCTGGTGG	TGCCGACAGA	TGGGCTGTAC	CTCATCTACT	CCCAGGTCCCT	8760
CTTCAGGGGC	CAAGGCTGCC	CTTCCACCAA	CGTTTTCTCT	ACTCACACCA	TCAGCCGCAT	8820
CGCCGTCTCC	TACCAGACCA	AGGTCAACCT	CCTCTCTGCC	ATCAAGAGCC	CTTGCCAGAG	8880
GGAGACCCCC	GAGGGGGCCG	AGGCCAAGCC	CTGGTACGAA	CCCATCTACC	TGGGAGGGGT	8940
CTTCCAGCTG	GAGAAGGATG	ATCGACTCAG	TGCCGAGATC	AACCTGCCCC	ACTATCTGGA	9000
CTTTGCTGAA	TCTGGGCAGG	TCTATTTTGG	GATCATTGCC	CTGTGAGGGG	GCAGGACATC	9060
CGTTCCCTCC	CCTGTCCATC	CCTTTATTAT	TTTACTCCTT	CAGACCCCCT	CACGTCCCTC	9120
TGGTTTAGAA	AGAGAAATGAG	GGGCTGGGGA	CTGGGCTCCA	AGCTTAAAC	TTTAAACAAC	9180
AACAGCAACA	CTTAGAAATC	AGGGATTTCAG	GGATGTGTGG	CCTGGACAAC	CAGGCACTGA	9240
CCACCACCAA	GAATTGGAAC	TGGGGCTTCC	AGACTCGCTG	GGGTCTTTGG	GTTTGGATTTC	9300
CTGGATGCAA	CCTGGGACAT	CTGGAATGTG	GCTGCCAGGG	AAGCTTGGGT	TCCAATCGGA	9360
ATACTCAGA	ACATTCCTTG	AGAAGATTTT	ACCTCAATCT	TGATGACTTT	TTAGGCTTCC	9420
CTTCTTCCA	ATTTTCCAGA	CTTCCCTGGG	ATGGGGAGCC	CAGCCCCAAA	CCCCACAGGC	9480
CAGCTCCCTC	TTATTTATAT	TTGCACTTGG	CATTATTATT	TATTTATTTA	TTTATTATTT	9540
ATTTACTAGT	GAATGTATTT	ATTCAGGAGG	GCGAGGTGTC	CTGGGAGACC	CAGCATAAGG	9600

(2) INFORMATION FOR SEQ ID NO: 12:

(A) LENGTH: 1644 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

CCTCAGCGAG	GACAGCAAGG	GACTAGCCAG	GAGGGAGAAC	AGAAACTCCA	GAACATCCTG	60
GAAATAGCTC	CCAGAAAAGC	AAGCAGCCAA	CCAGGCAGGT	TCTGTCCCTT	TCACTCACTG	120
GCCCAAGGCG	CCACATCTCC	CTCCAGAAAA	GACACCATGA	GCACAGAAAG	CATGATCCGC	180
GACGTGGAAC	TGGCAGAAGA	GGCACTCCCC	CAAAAGATGG	GGGGCTTCCA	GAACTCCAGG	240
CGGTGCCTAT	GTCTCAGCCT	CTTCTCATT	CTGCTTGTGG	CAGGGGCCAC	CACGCTCTTC	300
TGTCTACTGA	ACTTCGGGGT	GATCGGTCCC	CAAAGGGATG	AGAAGTTCCC	AAATGGCCTC	360
CCTCTCATCA	GTTCTATGGC	CCAGACCCTC	ACACTCAGAT	CATCTTCTCA	AAATTCGAGT	420
GACAAGCCTG	TAGCCCACGT	CGTAGCAAAC	CACCAAGTGG	AGGAGCAGCT	GGAGTGGCTG	480
AGCCAGCGCG	CCAACGCCCT	CCTGGCCAAC	GGCATGGATC	TCAAAGACAA	CCAAGTAGTG	540
GTGCCAGCCG	ATGGGTTGTA	CCTTGTCTAC	TCCCAGGTTT	TCTTCAAGGG	ACAAGGCTGC	600
CCCGACTACG	TGCTCCTCAC	CCACACCGTC	AGCCGATTTG	CTATCTCATA	CCAGGAGAAA	660
GTCAACCTCC	TCTCTGCCGT	CAAGAGCCCC	TGCCCCAAGG	ACACCCCTGA	GGGGGCTGAG	720
CTCAAACCTT	GGTATGAGCC	CATATACCTG	GGAGGAGTCT	TCCAGCTGGA	GAAGGGGGAC	780
CAACTCAGCG	CTGAGGTCAA	TCTGCCCAAG	TACTTAGACT	TTGCGGAGTC	CGGGCAGGTC	840
TACTTTGGAG	TCATTGCTCT	GTGAAGGGAA	TGGGTGTTCA	TCCATTCTCT	ACCCAGCCCC	900
CACCTTGACC	CCTTTACTCT	GACCCCTTTA	TTGTCTACTC	CTCAGAGCCC	CCAGTCTGTG	960
TCCTTCTAAC	TTAGAAAAGG	GATTATGGCT	CAGAGTCCAA	CTCTGTGCTC	AGAGCTTTCA	1020
ACAACATCTC	AGAAACACAA	GATGCTGGGA	CAGTGACCTG	GACTGTGGGC	CTCTCATGCA	1080
CCACCATCAA	GGACTCAAAT	GAGCCTTTCCG	AATTCAGTCT	AGCCTCGAAT	GTCCATTCTT	1140
GAGTTCCTGCA	AAGGGAGAGT	GGTCAGGTTG	CCTCTGTCTC	AGAATTGAGG	TGGATAAGAT	1200
CTCAGGCCTT	CCTACCTTCA	GACCTTTCCA	GACTCTTCCC	TGAGGTGCAA	TGCACAGCCT	1260
TCCTCACAGA	GCCAGCCCCC	CTCTATTTAT	ATTTGCACTT	ATTATTTATT	ATTTATTTAT	1320
TATTTATTTA	TTTGCTTATG	AATGTATTTA	TTTGGAAGGC	CGGGGTGTCC	TGGAGGACCC	1380
AGTGTGGGAA	GCTGTCTTCA	GACAGACATG	TTTTCTGTGA	AAACGGAGCT	GAGCTGTCCC	1440

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CACCTGGCCT	CTCTACCTTG	TTGCCTCCTC	TTTTGCTTAT	GTTTAAAACA	AAATATTTAT	1500
CTAACCCAAT	TGTCTTAATA	ACGCTGATTT	GGTGACCAGG	CTGTCGCTAC	ATCACTGAAC	1560
CTCTGCTCCC	CACGGGAGCC	GTGACTGTAA	TTGCCCTACA	GTCAATTGAG	AGAAATAAAG	1620
ATCGCTTAAA	ATAAAAAACC	CCCC				1644

## (2) INFORMATION FOR SEQ ID NO: 13:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1890 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

AAACAGAGAG	AGATAGAGAA	AGAGAAAGAC	AGAGGTGTTT	CCCTTAGCTA	TGGAAACTCT	60
ATAAGAGAGA	TCCAGCTTGC	CTCCTCTTGA	GCAGTCAGCA	ACAGGGTCCC	GTCCTTGACA	120
CCTCAGCCTC	TACAGGACTG	AGAAGAAGTA	AAACCGTTTG	CTGGGGCTGG	CCTGACTCAC	180
CAGCTGCCAT	GCAGCAGCCC	TTCAATTACC	CATATCCCCA	GATCTACTGG	GTGGACAGCA	240
GTGCCAGCTC	TCCCTGGGCC	CCTCCAGGCA	CAGTTCTTCC	CTGTCCAACC	TCTGTGCCCA	300
GAAGGCCTGG	TCAAAGGAGG	CCACCACCAC	CACCGCCACC	GCCACCACTA	CCACCTCCGC	360
CGCCGCCGCC	ACCACTGCCT	CCACTACCGC	TGCCACCCCT	GAAGAAGAGA	GGGAACCACA	420
GCACAGGCCT	GTGTCTCCTT	GTGATGTTTT	TCATGGTTCT	GGTTGCCTTG	GTAGGATTGG	480
GCCTGGGGAT	GTTTCAGCTC	TTCCACCTAC	AGAAGGAGCT	GGCAGAACTC	CGAGAGTCTA	540
CCAGCCAGAT	GCACACAGCA	TCATCTTTGG	AGAAGCAAAT	AGGCCACCCC	AGTCCACCCC	600
CTGAAAAAAA	GGAGCTGAGG	AAAGTGGCCC	ATTTAACAGG	CAAGTCCAAC	TCAAGGTCCA	660
TGCCTCTGGA	ATGGGAAGAC	ACCTATGGAA	TTGTCCTGCT	TTCTGGAGTG	AAGTATAAGA	720
AGGGTGGCCT	TGTGATCAAT	GAAACTGGGC	TGTACTTTGT	ATATTCCAAA	GTATACTTCC	780
GGGGTCAATC	TTGCAACAAC	CTGCCCCTGA	GCCACAAGGT	CTACATGAGG	AACTCTAAGT	840
ATCCCCAGGA	TCTGGTGATG	ATGGAGGGGA	AGATGATGAG	CTACTGCACT	ACTGGGCAGA	900
TGTGGGCCCC	CAGCAGCTAC	CTGGGGGCAG	TGTTCAATCT	TACCAGTGCT	GATCATTTAT	960
ATGTCAACGT	ATCTGAGCTC	TCTCTGGTCA	ATTTTGAGGA	ATCTCAGACG	TTTTTCGGCT	1020
TATATAAGCT	CTAAGAGAAG	CACTTTGGA	TTCTTTCCAT	TATGATTCTT	TGTTACAGGC	1080
ACCGAGAATG	TTGTATTGAG	TGAGGGTCTT	CTTACATGCA	TTTGAGGTCA	AGTAAGAAGA	1140
CATGAACCAA	GTGGACCTTG	AGACCACAGG	GTTCAAAAATG	TCTGTAGCTC	CTCAACTCAC	1200
CTAATGTTTA	TGAGCCAGAC	AAATGGAGGA	ATATGACGGA	AGAACATAGA	ACTCTGGGCT	1260
GCCATGTGAA	GAGGGAGAAG	CATGAAAAAG	CAGCTACCCA	GGTGTCTTAC	ACTCATCTTA	1320
GTGCCCTGAGA	GTATTTAGGC	AGATTGAAAA	GGACACCTTT	TAACCTCACCT	CTCAAGGTGG	1380
GCCTTGCTAC	CTCAAGGGGG	ACTGTCTTTC	AGATACATGG	TTGTGACCTG	AGGATTTAAG	1440
GGATGGAAAA	GGAAGACTAG	AGGCTTGCAAT	AATAAGCTAA	AGAGGCTGAA	AGAGGCCAAT	1500
GCCCCACTGG	CAGCATCTTC	ACTTCTAAAT	GCATATCCTG	AGCCATCGGT	GAAACTAACA	1560
GATAAGCAAG	AGAGATGTTT	TGGGGACTCA	TTTCATTCCCT	AACACAGCAT	GTGTATTTCC	1620
AGTGCCAATT	GTAGGGGTGT	GTGTGTGTGT	GTGTGTGTGT	GTGTATGACT	AAAGAGAGAA	1680
TGTAGATATT	GTGAAGTACA	TATTAGGAAA	ATATGGGTTG	CATTGTGTCA	AGATTTTGAA	1740
TGCTTCCTGA	CAATCAACTC	TAATAGTGCT	TAAAAATCAT	TGATTGTGAG	CTACTAATGA	1800
TGTTTTCCCTA	TAATATAATA	AATATTTATG	TAGATGTGCA	TTTTTGTGAA	ATGAAAACAT	1860
GTAATAAAAA	GTATATGTTA	GGATACAAAT				1890

## (2) INFORMATION FOR SEQ ID NO: 14:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1541 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

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GGGTGTCTCA CAGAGAAGCA AAGAGAAGAG AACAGGAGAA ATGGTGTTTC CCTTGACTGC      60
GGAAACTTTA TAAAGAAAAC TTAGCTTCTC TGGAGCAGTC AGCGTCAGAG TTCTGTCCTT      120
GACACCTGAG TCTCCTCCAC AAGGCTGTGA GAAGGAAACC CTTTCCTGGG GCTGGGTGCC      180
ATGCAGCAGC CCATGAATTA CCCATGTCCC CAGATCTTCT GGGTAGACAG CAGTGCCACT      240
TCATCTTGGG CTCCTCCAGG GTCAGTTTTT CCCTGTCCAT CTTGTGGGCC TAGAGGGCCG      300
GACCAAAGGA GACCGCCACC TCCACCACCA CCTGTGTCAC CACTACCACC GCCATCACAA      360
CCACTCCCAC TGCCGCCACT GACCCCTCTA AAGAAGAAGG ACCACAACAC AAATCTGTGG      420
CTACCGGTGG TATTTTTTCAT GGTTCGTGGT GCTCTGGTTG GAATGGGATT AGGAATGTAT      480
CAGCTCTTCC ACCTGCAGAA GGAAGTGGCA GAACTCCGTG AGTTCACCAA CCAAAGCCTT      540
AAAGTATCAT CTTTTGAAAA GCAAATAGCC AACCCTAGTA CACCCTCTGA AAAAAAGAG      600
CCGAGGAGTG TGGCCCATTT AACAGGGAAC CCCCCTCAA GGTCCATCCC TCTGGAATGG      660
GAAGACACAT ATGGAACCGC TCTGATCTCT GGAGTGAAGT ATAAGAAAGG TGGCCTTGTTG      720
ATCAACGAAA CTGGGTTGTA CTTCTGTGAT TCCAAAGTAT ACTTCCGGGG TCAGTCTTGC      780
AACAACCAGC CCCTAAACCA CAAGGTCTAT ATGAGGAACT CTAAGTATCC TGAGGATCTG      840
GTGCTAATGG AGGAGAAGAG GTTGAAGTAC TGCACTACTG GCCAGATATG GGCCACAGC      900
AGCTACCTGG GGGCAGTATT CAATCTTACC AGTGCTGACC ATTTATATGT CAACATATCT      960
CAACTCTCTC TGATCAATTT TGAGGAATCT AAGACCTTTT TCGGCTTGTA TAAGCTTTAA      1020
AAGAAAAAGC ATTTTAAAT GATCTACTAT TCTTTATCAT GGGCACCAGG AATATTGTCT      1080
TGAATGAGAG TCTTCTTAAG ACCTATTGAG ATTAATTAAG ACTACATGAG CCACAAAGAC      1140
CTCATGACCG CAAGGTCCAA CAGGTCAGCT ATCCTTCATT TTCTCGAGGT CCATGGAGTG      1200
GTCCTTAATG CCTGCATCAT GAGCCAGATG GAAGGAGGTC TGTGACTGAG GGACATAAAG      1260
CTTTGGGCTG CTGTGTAGCA ATGCAGAGGC ACAGAGAAAG AACTGTCTGA TGTAAATGG      1320
CCAAGAGAA TTTAACCATT GAAGAAGACA CCTTTACACT CACTCCAGG GTGGGTCTAC      1380
TTACTACCTC ACAGAGGCCG TTTTGTAGAC ATAGTTGTGG TATGAATATA CAAGGGTGAG      1440
AAAGGAGGCT CATTTGACTG ATAAGCTAGA GACTGAAAAA AAGACAGTGT CTCATTGGCA      1500
CCATCTTTAC TGTACCTGA TGTTCCTGA GCCGACCTTT G      1541

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## (2) INFORMATION FOR SEQ ID NO: 15:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 888 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GGCTGGTCCC	CTGACAGGTT	GAAGCAAGTA	GACGCCCAGG	AGCCCCGGGA	GGGGGCTGCA	60
GTTTCCTTCC	TTCCTTCTCG	GCAGCGCTCC	GCGCCCCCAT	CGCCCTCCT	GCGCTAGCGG	120
AGGTGATCGC	CGCGGCGATG	CCGGAGGAGG	GTTCCGGGCTG	CTCGGTGCGG	CGCAGGCCCT	180
ATGGGTGCGT	CCTGCGGGCT	GCTTTGGTCC	CATTGGTCGC	GGGCTTGGTG	ATCTGCCTCG	240
TGGTGTGCAT	CCAGCGCTTC	GCACAGGCTC	AGCAGCAGCT	GCCGCTCGAG	TCACTTGGGT	300
GGGACGTAGC	TGAGCTGCAG	CTGAATCACA	CAGGACCTCA	GCAGGACCCC	AGGCTATACT	360
GGCAGGGGGG	CCCAGCACTG	GGCCGCTCCT	TCCTGCATGG	ACCAGAGCTG	GACAAGGGGC	420
AGCTACGTAT	CCATCGTGAT	GGCATCTACA	TGGTACACAT	CCAGGTGACG	CTGGCCATCT	480
GCTCCTCCAC	GACGGCCTCC	AGGCACCACC	CCACCACCCT	GGCCGTGGGA	ATCTGCTCTC	540
CCGCCTCCCG	TAGCATCAGC	CTGCTGCGTC	TCAGTTTCCA	CCAAGTTGT	ACCATTGCCT	600
CCCAGCGCCT	GACGCCCCTG	GCCCCGAGGG	ACACACTCTG	CACCAACCTC	ACTGGGACAC	660
TTTTGCCTTC	CCGAAACACT	GATGAGACCT	TCTTTGGAGT	GCACTGGGTG	CGCCCTGAC	720
CACTGCTGCT	GATTAGGGTT	TTTTAAATTT	TATTTTATTT	TATTTAAGTT	CAAGAGAAAA	780
AGTGTACACA	CAGGGGCCAC	CCGGGGTTGG	GGTGGGAGTG	TGGTGGGGGG	TAGTGGTGGC	840
AGGACAAGAG	AAGGCATTGA	GCTTTTTCTT	TCATTTTCCT	ATTAAAAA		888

## (2) INFORMATION FOR SEQ ID NO: 16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1906 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CCAAGTCACA	TGATTCAGGA	TTCAGGGGGA	GAATCCTTCT	TGGAACAGAG	ATGGGCCCAG	60
AACTGAATCA	GATGAAGAGA	GATAAGGTGT	GATGTGGGGA	AGACTATATA	AAGAATGGAC	120
CCAGGGCTGC	AGCAAGCACT	CAACGGAATG	GCCCCCTCCTG	GAGACACAGC	CATGCATGTG	180
CCGCGGGGCT	CCGTGGCCAG	CCACCTGGGG	ACCACGAGCC	GCAGCTATTT	CTATTTGACC	240
ACAGCCACTC	TGGCTCTGTG	CCTTGTCTTC	ACGGTGGCCA	CTATTATGGT	GTTGGTCGTT	300
CAGAGGACGG	ACTCCATTCC	CAACTCACCT	GACAACGTCC	CCCTCAAAGG	AGGAAATTGC	360
TCAGAAGACC	TCTTATGTAT	CCTGAAAAGA	GCTCCATTCA	AGAAGTCATG	GGCCTACCTC	420
CAAGTGGCAA	AGCATCTAAA	CAAAACCAAG	TTGTCTTGGA	ACAAAGATGG	CATTCTCCAT	480
GGAGTCAGAT	ATCAGGATGG	GAATCTGGTG	ATCCAATTCC	CTGGTTTGTA	CTTCATCATT	540
TGCCAACTGC	AGTTTCTTGT	ACAATGCCCA	AATAATTCTG	TCGATCTGAA	GTTGGAGCTT	600
CTCATCAACA	AGCATATCAA	AAAACAGGCC	CTGGTGACAG	TGTGTGAGTC	TGGAATGCAA	660
ACGAAACACG	TATACCAGAA	TCTCTCTCAA	TTCTTGCTGG	ATTACCTGCA	GGTCAACACC	720
ACCATATCAG	TCAATGTGGA	TACATTCCAG	TACATAGATA	CAAGCACCTT	TCCTCTTGAG	780
AATGTGTTGT	CCATCTTCTT	ATACAGTAAT	TCAGACTGAA	CAGTTTCTCT	TGGCCTTCAG	840
GAAGAAAGCG	CCTCTCTACC	ATACAGTATT	TCATCCCTCC	AAACACTTGG	GCAAAAAGAA	900
AACTTTAGAC	CAAGACAAAC	TACACAGGGT	ATTAAATAGT	ATACTTCTCC	TTCTGTCTCT	960
TGGAAGATA	CAGCTCCAGG	GTTAAAAAGA	GAGTTTTTAG	TGAAGTATCT	TTCAGATAGC	1020
AGGCAGGGAA	GCAATGTAGT	GTGGTGGGCA	GAGCCCCACA	CAGAATCAGA	AGGGATGAAT	1080
GGATGTCCCA	GCCCAACCAC	TAATTCACCTG	TATGGTCTTG	ATCTATTTCT	TCTGTTTTGA	1140

(2) INFORMATION FOR SEO ID NO: 17:

(A) LENGTH: 1619 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

GT CATGGAAT	ACGCCTCTGA	CGCTTCACTG	GACCCCGAAG	CCCCGTGGCC	TCCCGCGCCC	60
CGCGCTCGCG	CCTGCCGCGT	ACTGCCTTGG	GCCCTGGTCG	CGGGGCTGCT	GCTGCTGCTG	120
CTGCTCGCTG	CCGCCTGCGC	CGTCTTCCTC	GCCTGCCCCT	GGGCCGTGTC	CGGGGCTCGC	180
GCCTCGCCCCG	GCTCCGCGGC	CAGCCCGAGA	CTCCGCGAGG	GTCCCGAGCT	TTCGCCCCGAC	240
GATCCCGCCG	GCCTCTTGGA	CCTGCGGCAG	GGCATGTTTG	CGCAGCTGGT	GGCCCAAAT	300
GTTCTGCTGA	TCGATGGGCC	CCTGAGCTGG	TACAGTGACC	CAGGCCTGGC	AGGCGTGTCC	360
CTGACGGGGG	GCCTGAGCTA	CAAAGAGGAC	ACGAAGGAGC	TGGTGGTGGC	CAAGGCTGGA	420
GTCTACTATG	TCTTCTTTCA	ACTAGAGCTG	CGGCGCGTGG	TGGCCGCGCA	GGGCTCAGGC	480
TCCGTTTCAC	TTGCGCTGCA	CCTGCAGCCA	CTGCGCTCTG	CTGCTGGGGC	CGCCGCCCTG	540
GCTTTGACCG	TGGACCTGCC	ACCCGCCTCC	TCCGAGGCTC	GGAACTCGGC	CTTCGTTTTC	600
CAGGGCCGCT	TGCTGCACCT	GAGTGCCGGC	CAGCGCCTGG	GCGTCCATCT	TCACACTGAG	660
GCCAGGGCAC	GCCATGCCTG	GCAGCTTACC	CAGGGCGCCA	CAGTCTTGGG	ACTCTTCCGG	720
GTGACCCCCG	AAATCCCAGC	CGGACTCCCT	TCACCGAGGT	CGGAATAACG	CCCAGCCTGG	780
GTGCAGCCCA	CCTGGACAGA	GTCCGAATCC	TACTCCATCC	TTCATGGAGA	CCCCTGGTGC	840
TGGGTCCCCTG	CTGCTTTCTC	TACCTCAAGG	GGCTTGGCAG	GGGTCCCTGC	TGCTGACCTC	900
CCCTTGAGGA	CCCTCCTCAC	CCACTCCTTC	CCCAAGTTGG	ACCTTGATAT	TTATTCTGAG	960
CCTGAGCTCA	GATAATATAT	TATATATATT	ATATATATAT	ATATATTTCT	ATTTAAAGAG	1020
GATCTTGAGT	TTGTTGAATGG	ACTTTTTTAG	AGGAGTTGTT	TTGGGGGGGG	GGTCTTCGAC	1080
ATTGCCGAGG	CTGGTCTTGA	ACTCCTGGAC	TTAGACGATA	CTCCTGCCTC	AGCCTCCCAA	1140
GCAACTGGGA	TTCATCCTTT	CTATTAATTC	ATTGTACTTA	TTTGCCTATT	TGTGTGTATT	1200
GAGCATCTGT	AATGTGCCAG	CATTGTGCCC	AGGCTAGGGG	GCTATAGAAA	CATCTAGAAA	1260
TAGACTGAAA	GAAAATCTGA	GTTATGGTAA	TACGTGAGGA	ATTTAAAGAC	TCATCCCCAG	1320
CCTCCACCTC	CTGTGTGATA	CTTGGGGGCT	AGCTTTTTTTC	TTTCTTTTCTT	TTTTTTTGAGA	1380

[illegible]



TGGTCTTGTT	CTGTCAACCA	GGCTAGAATG	CAGCGGTGCA	ATCATGAGTC	AATGCAGCCT	1440
CCAGCCTCGA	CCTCCCGAGG	CTCAGGTGAT	CCTCCCATCT	CAGCCTCTCG	AGTAGCTGGG	1500
ACCACAGTTG	TGTGCCACCA	CACCTGGCTA	ACTTTTTAAT	TTTTTTGCGG	AGACGGTATT	1560
GCTATGTTGC	CAAGGTTGTT	TACATGCCAG	TACAATTTAT	AATAAACACT	CATTTTTTCC	1619

## (2) INFORMATION FOR SEQ ID NO: 18:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1239 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AGCCTATAAA	GCACGGGCAC	TGGCGGGAGA	CGTGCACTGA	CCGACCGTGG	TAATGGACCA	60
GCACACACTT	GATGTGGAGG	ATACCGCGGA	TGCCAGACAT	CCAGCAGGTA	CTTCGTGCCC	120
CTCGGATGCG	GCGCTCCTCA	GAGATACCGG	GCTCCTCGCG	GACGCTGCGC	TCCTCTCAGA	180
TACTGTGCGC	CCCACAAATG	CCGCGCTCCC	CACGGATGCT	GCCTACCTTG	CGGTTAATGT	240
TCGGGATCGC	GAGGCCGCGT	GGCCGCCTGC	ACTGAACTTC	TGTTCCCGCC	ACCCAAAGCT	300
CTATGGCCTA	GTCGCTTTGG	TTTTGCTGCT	TCTGATCGCC	GCCTGTGTTC	CTATCTTCAC	360
CCGCACCGAG	CCTCGGCCAG	CGCTCACAAT	CACCACCTCG	CCCAACCTGG	GTACCCGAGA	420
GAATAATGCA	GACCAGGTCA	CCCCTGTTTC	CCACATTGGC	TGCCCAACA	CTACACAACA	480
GGGCTCTCCT	GTGTTCGCCA	AGCTACTGGC	TAAAAACCAA	GCATCGTTGT	GCAATACAAC	540
TCTGAACTGG	CACAGCCAAG	ATGGAGCTGG	GAGCTCATAC	CTATCTCAAG	GTCTGAGGTA	600
CGAAGAAGAC	AAAAAGGAGT	TGGTGGTAGA	CAGTCCCGGG	CTCTACTACG	TATTTTTTGA	660
ACTGAAGCTC	AGTCCAACAT	TCACAAACAC	AGGCCACAAG	GTGCAGGGCT	GGGTCTCTCT	720
TGTTTTGCAA	GCAAAGCCTC	AGGTAGATGA	CTTTGACAAC	TTGGCCCTGA	CAGTGGAACT	780
GTTCCCTTGC	TCCATGGAGA	ACAAGTTAGT	GGACCGTTCC	TGGAGTCAAC	TGTTGCTCCT	840
GAAGGCTGGC	CACCGCCTCA	GTGTGGGTCT	GAGGGCTTAT	CTGCATGGAG	CCCAGGATGC	900
ATACAGAGAC	TGGGAGCTGT	CTTATCCCAA	CACCACCAGC	TTTGGACTCT	TTCTTGTGAA	960
ACCCGACAAC	CCATGGGAAT	GAGAACTATC	CTTCTTGTGA	CTCCTAGTTG	CTAAGTCCTC	1020
AAGCTGCTAT	GTTTATGGG	GTCTGAGCAG	GGGTCCCTTC	CATGACTTTC	TCTTGTCTTT	1080
AAGTGGACTT	GGTATTTATT	CTGAGCATAG	CTCAGACAAG	ACTTTATATA	ATTCAGTAGA	1140
TAGCATTAGT	AAACTGCTGG	GCAGCTGCTA	GATAAAAAAA	AATTTCTAAA	TCAAAGTTTA	1200
TATTTATATT	AATATATAAA	AATAAATGTG	TTTGTAAT			1239

## (2) INFORMATION FOR SEQ ID NO: 19:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 606 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATGATCGAAA	CATACAACCA	AACTTCTCCC	CGATCTGCGG	CCACTGGACT	GCCCATCAGC	60
ATGAAAATTT	TTATGTATTT	ACTTACTGTT	TTTCTTATCA	CCCAGATGAT	TGGGTCAGCA	120
CTTTTGTCTG	TGTATCGCTT	CGCACAGGCT	TTTGAAATGC	AAAAAGGTGA	TCAGAATCCT	180
CAAATTGCGG	CACATGTCAT	AAGTGAGGCC	AGCAGTAAAA	CAACATCTGT	GTTACAGTGG	240
GCTGAAAAAG	GATACTACAC	CATGAGCAAC	AACTTGGTAA	CCCTGGAAAA	TGGGAAACAG	300
CTGACCGTTA	AAAGACAAGG	ACTCTATTAT	ATCTATGCCC	AAGTCACCTT	CTGTTCCAAT	360
CGGGAAGCTT	CGAGTCAAGC	TCCATTTATA	GCCAGCCTCT	GCCTAAAGTC	CCCCGGTAGA	420
TTGAGAGAAA	TCTTACTCAG	AGCTGCAAAT	ACCCACAGTT	CCGCCAAACC	TTGCGGGCAA	480
CAATCCATTC	ACTTGGGAGG	AGTATTTGAA	TTGCAACCAG	GTGCTTCGGT	GTTTGTCAAT	540
GTGACTGATC	CAAGCCAAGT	GAGCCATGGC	ACTGGCTTCA	CGTCCTTTGG	CTTACTCAA	600
CTCTGA						606

## (2) INFORMATION FOR SEQ ID NO: 20:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 783 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

ATGATCGAAA	CATACAACCA	AACTTCTCCC	CGATCTGCGG	CCACTGGACT	GCCCATCAGC	60
ATGAAAATTT	TTATGTATTT	ACTTACTGTT	TTTCTTATCA	CCCAGATGAT	TGGGTCAGCA	120
CTTTTGTCTG	TGTATCTTCA	TAGAAGATTG	GATAAGGTCG	AAGAGGAAGT	AAACCTTCAT	180
GAAGATTTTG	TATTCATAAA	AAAGCTAAAG	AGATGCAACA	AAGGAGAAGG	ATCTTTATCC	240
TTGCTGAACT	GTGAGGAGAT	GAGAAGGCAA	TTTGAAGACC	TTGTCAAGGA	TATAACGTTA	300
AACAAAGAAG	AGAAAAAAGA	AAACAGCTTT	GAAATGCAAA	AAGGTGATCA	GAATCCTCAA	360
ATTGCGGCAC	ATGTCATAAG	TGAGGCCAGC	AGTAAACAA	CATCTGTGTT	ACAGTGGGCT	420
GAAAAAGGAT	ACTACACCAT	GAGCAACAAC	TTGGTAACCC	TGGAAAATGG	GAAACAGCTG	480
ACCGTTAAAA	GACAAGGACT	CTATTATATC	TATGCCCAAG	TCACCTTCTG	TTCCAATCGG	540
GAAGCTTCGA	GTCAAGCTCC	ATTTATAGCC	AGCCTCTGCC	TAAAGTCCCC	CGGTAGATTG	600
GAGAGAATCT	TACTCAGAGC	TGCAAATACC	CACAGTTCCG	CCAAACCTTG	CGGGCAACAA	660
TCCATTCACT	TGGGAGGAGT	ATTTGAATTG	CAACCAGGTG	CTTCGGTGTT	TGTCAATGTG	720
ACTGATCCAA	GCCAAGTGAG	CCATGGCACT	GGCTTCACGT	CCTTTGGCTT	ACTCAAATC	780
TGA						783

## (2) INFORMATION FOR SEQ ID NO: 21:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 558 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTGCTGCACT	TCGGGGTAAT	CGGCCCCCAG	AGGGAAGAGC	AGTCCCCAGG	TGGCCCCCTCC	60
ATCAACAGCC	CTCTGGTTCA	AACACTCAGG	TCCTCTTCTC	AAGCCTCAAG	TAACAAGCCG	120
GTAGCCACG	TTGTAGCCGA	CATCAACTCT	CCGGGGCAGC	TCCGGTGGTG	GGACTCGTAT	180
GCCAATGCCC	TCATGGCCAA	CGGTGTGAAG	CTGGAAGACA	ACCAGCTGGT	GGTGCCTGCT	240
GACGGGCTTT	ACCTCATCTA	CTCACAGGTC	CTCTTCAGGG	GCCAAGGCTG	CCCTTCCACC	300
CCCTTGTTCC	TCACCCACAC	CATCAGCCGC	ATTGCAGTCT	CCTACCAGAC	CAAGGTCAAC	360
ATCCTGTCTG	CCATCAAGAG	CCCTTGCCAC	AGGGAGACCC	CAGAGTGGGC	TGAGGCCAAG	420
CCCTGGTACG	AACCCATCTA	CCAGGGAGGA	GTCTTCCAGC	TGGAGAAGGG	AGATCGCCTC	480
AGTGCTGAGA	TCAACCTGCC	GGACTACCTG	GACTATGCCG	AGTCCGGGCA	GGTCTACTTT	540
GGGATCATTG	CCCTGTGA					558

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1783 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CAAGTCACAT	GATCCAGGAT	GCAGGGGAAA	ATCCTTCTTG	GAACAGAGCT	GGGTACAGAA	60
CCGAATCAGA	TGAGGAGAGA	TAAGGTGTGA	TGTGGGACAG	ACTATATAAA	GCATGGAGCC	120
AGGGCTGCAA	CAAGCAGGCA	GCTGTGGGGC	TCCTTCCCCT	GACCCAGCCA	TGCAGGTGCA	180
GCCCCGGCTCG	GTAGCCAGCC	CCTGGAGAAG	CACGAGGCC	TGGAGAAGCA	CAAGTCGCAG	240
CTACTTCTAC	CTCAGCACCA	CCGCACTGGT	GTGCCTTGTT	GTGGCAGTGG	CGATCATTTCT	300
GGTACTGGTA	GTCCAGAAAA	AGGACTCCAC	TCCAAATACA	ACTGAGAAGG	CCCCCCTTAA	360
AGGAGGAAAT	TGCTCAGAGG	ATCTCTTCTG	TACCTTGAAA	AGTACTCCAT	CCAAGAAGTC	420
ATGGGCGCTAC	CTCCAAGTGT	CAAAGCATCT	CAACAATACC	AAACTGTCAT	GGAACGAAGA	480
TGGCACCATC	CACGGACTCA	TATACCAGGA	CGGGAACCTG	ATAGTCCAAT	TCCCTGGCTT	540
GTACTTCATC	GTTTGCCAAC	TGCAGTTCCT	CGTGCAGTGC	TCAAATCATT	CTGTGGACCT	600
GACATTGCAG	CTCCTCATCA	ATTCCAAGAT	CAAAAAGCAG	ACGTTGGTAA	CAGTGTGTGA	660
GTCTGGAGTT	CAGAGTAAGA	ACATCTACCA	GAATCTCTCT	CAGTTTTTGC	TGCATTACTT	720
ACAGGTCAAC	TCTACCATAT	CAGTCAGGGT	GGATAATTTT	CAGTATGTGG	ATACAAACAC	780
TTTCCCTCTT	GATAATGTGC	TATCCGTCTT	CTTATATAGT	AGCTCAGACT	GAATAGTTGT	840
TCTTAACCTT	TATGAAAATG	CTGTCTACCA	TACAGTACTT	CATCTGTCCA	AACATGGGCC	900
AAAGAAAATA	TTAGGACAAC	TCAAACCTAAG	CATGTGAGTT	AGTGCACTTC	TCTTTCTGTC	960
CTTTGGAAAA	ATACAAACCC	AGGATTTAGA	AAGTGGAGTC	TCCTTCAGAT	GCACAAACAG	1020
GAAAGAATGT	GATATGTGCA	CAGAGACCTA	CTTGGGCACT	AGAAGGGGTG	TGAGTTGTCC	1080
CAGTATAACC	ACTAATTCAC	TGACCTTGAG	CCATTTTTCC	TTCCCCCTGG	AACTTGGGGT	1140
CTGAATCTGG	AAAAGTAGGA	GATGAGATTT	ACATTTCCCC	AATATTTTCT	TCAACTCAGA	1200
AGACGAGACT	GTGGAGCTGA	GCTCCCTACA	CAGATGAAGG	CCTCCCATGG	CATGAGGAAA	1260

ATGATGGTAC	CAGTAATGTC	TGTCTGACTG	TCATCTCAGC	AAGTCCTAAG	GACTTCCATG	1320
CTGCCTTGTT	GAAAGATACT	CTAACCTCTT	GTAATGGGCA	AAGTGATCCT	GTCTCTCACT	1380
GAGGGGAGTA	GCTGCTGCCA	TCTCCTGAGA	CATACATGGA	GACATTTTCT	GCCCAAATTC	1440
CATTCTGTGT	GCAGTTTTTA	AGTATTCCCC	CAAAAGTTCT	TGACAATGAG	AACTTTGAAT	1500
GTGGGAAGAG	CTTCTGGACA	GCAAACATTA	ACAGCTTCTC	CTGACCAGAG	AGACCATGCA	1560
AGCTTGGTCT	TAGACCCATC	AAGCTTGAGG	TTTCTACATT	GTGGGAGACA	GACTTTTGAC	1620
AAACCATTTG	AGTTGATGTC	TGGGCCCCTG	GGAGTTCTCC	TTCAAGTAAGG	AGAGCAAGCC	1680
GTTCTAGTGC	TGTGTCAGAG	GATGGAGTAA	AATAGACACT	TTTCTGAAGG	AAAGGAGAAC	1740
AAAGTTCCAG	AAAAAGGCTA	GAAAATGTTT	AAAAAGAAAA	AAA		1783

## (2) INFORMATION FOR SEQ ID NO: 23:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1047 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

AGAGAGCGCT	GGGAGCCGGA	GGGGAGCGCA	GCGAGTTTTG	GCCAGTGGTC	GTGCAGTCCA	60
AGGGGCTGGA	TGGCATGCTG	GACCCAAGCT	CAGCTCAGCG	TCCGGACCCA	ATAACAGTTT	120
TACCAAGGGA	GCAGCTTTCT	ATCCTGGCCA	CACTGAGGTG	CATAGCGTAA	TGTCCATGTT	180
GTTCTACACT	CTGATCACAG	CTTTTCTGAT	CGGCATACAG	GCGGAACCAC	ACTCAGAGAG	240
CAATGTCCCT	GCAGGACACA	CCATCCCCCA	AGTCCACTGG	ACTAAACTTC	AGCATTC CCT	300
TGACACTGCC	CTTCGCAGAG	CCCGCAGCGC	CCCGGCAGCG	GCGATAGCTG	CACGCGTGGC	360
GGGGCAGACC	CGCAACATTA	CTGTGGACCC	CAGGCTGTTT	AAAAAGCGGC	GACTCCGTTC	420
ACCCCGTGTG	CTGTTTAGCA	CCCAGCCTCC	CCGTGAAGCT	GCAGACACTC	AGGATCTGGA	480
CTTCGAGGTC	GGTGGTGCTG	CCCCTTTCAA	CAGGACTCAC	AGGAGCAAGC	GGTCATCATC	540
CCATCCCATC	TTCCACAGGG	GCGAATTCTC	GGTGTGTGAC	AGTGT CAGCG	TGTGGGTTGG	600
GGATAAGACC	ACCGCCACAG	ACATCAAGGG	CAAGGAGGTG	ATGGTGTGG	GAGAGGTGAA	660
CATTAACAAC	AGTGTATTCA	AACAGTACTT	TTTTGAGACC	AAGTGCCGGG	ACCCAAATCC	720
CGTTGACAGC	GGGTGCCGGG	GCATTGACTC	AAAGCACTGG	AACTCATATT	GTACCACGAC	780
TCACACCTTT	GTCAAGGCGC	TGACCATGGA	TGGCAAGCAG	GCTGCCTGGC	GGTTTATCCG	840
GATAGATACG	GCCTGTGTGT	GTGTGCTCAG	CAGGAAGGCT	GTGAGAAGAG	CCTGACCTGC	900
CGACACGCTC	CCTCCCCCTG	CCCCTTCTAC	ACTCTCCTGG	GCCCCCTCCT	ACCTCAACCT	960
GTAAATTATT	TTAAATTATA	AGGACTGCAT	GGTAATTTAT	AGTTTATACA	GTTTTTAAAGA	1020
ATCATTATTT	ATTAAATTTT	TGGAAGC				1047

## (2) INFORMATION FOR SEQ ID NO: 24:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1176 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GAGCGCCTGG	AGCCGGAGGG	GAGCGCATCG	AGTGACTTTG	GAGCTGGCCT	TATATTTGGA	60
TCTCCCGGGC	AGCTTTTTTG	AAACTCCTAG	TGAACATGCT	GTGCCCTCAAG	CCAGTGAAAT	120
TAGGCTCCCT	GGAGGTGGGA	CACGGGCAGC	ATGGTGGAGT	TTTGGCCTGT	GGTCGTGCAG	180
TCCAGGGGGC	TGGATGGCAT	GCTGGACCCA	AGCTCACCTC	AGTGTCTGGG	CCCAATAAAG	240
GTTTTGCCAA	GGACGCAGCT	TTCTATACTG	GCCGCAGTGA	GGTGCATAGC	GTAATGTCCA	300
TGTTGTTCTA	CACTCTGATC	ACTGCGTTTT	TGATCGGCGT	ACAGGCAGAA	CCGTACACAG	360
ATAGCAATGT	CCCAGAAGGA	GACTCTGTCC	CTGAAGCCCA	CTGGACTAAA	CTTCAGCATT	420
CCCTTGACAC	AGCCCTCCGC	AGAGCCCGCA	GTGCCCCCTAC	TGCACCAATA	GCTGCCCGAG	480
TGACAGGGCA	GACCCGCAAC	ATCACTGTAG	ACCCCGAGCT	GTTTAAGAAA	CGGAGACTCC	540
ACTCACCCCG	TGTGCTGTTT	AGCACCCAGC	CTCCACCCAC	CTCTTCAGAC	ACTCTGGATC	600
TAGACTTCCA	GGCCCATGGT	ACAATCCCTT	TCAACAGGAC	TCACCGGAGC	AAGCGCTCAT	660
CCACCCACCC	AGTCTTCCAC	ATGGGGGAGT	TCTCAGTGTG	TGACAGTGTG	AGTGTGTGGG	720
TTGGAGATAA	GACCACAGCC	ACAGACATCA	AGGGCAAGGA	GGTGACAGTG	CTGGCCGAGG	780
TGAACATTAA	CAACAGTGTA	TTCAGACAGT	ACTTTTTTTGA	GACCAAGTGC	CGAGCCTCCA	840
ATCCTGTTGA	GAGTGGGTGC	CGGGGCATCG	ACTCCAAACA	CTGGAACTCA	TACTGCACCA	900
CGACTCACAC	CTTCGTCAAG	GCGTTGACAA	CAGATGAGAA	GCAGGCTGCC	TGGAGGTTCA	960
TCCGGATAGA	CACAGCCTGT	GTGTGTGTGC	TCAGCAGGAA	GGCTACAAGA	AGAGGCTGAC	1020
TTGCCTGCAG	CCCCCTTCCC	CACCTGCCCC	CTCCACACTC	TCTTGGGCCC	CTCCCTACCT	1080
CAGCCTGTAA	ATTATTTTAA	ATTATAAGGA	CTGCATGATA	ATTTATCGTT	TATACAATTT	1140
TAAAGACATT	ATTTATTAAA	TTTTCAAAGC	ATCCTG			1176

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1623 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

TCAGAGTCCT	GTCCTTGACA	CTTCAGTCTC	CACAAGACTG	AGAGGAGGAA	ACCCTTTCCT	60
GGGGCTGGGT	GCCATGCAGC	AGCCCGTGAA	TTACCCATGT	CCCCAGATCT	ACTGGGTAGA	120
CAGCAGTGCC	ACTTCTCCTT	GGGCTCCTCC	AGGGTCAGTT	TTTTCTTGTC	CATCCTCTGG	180
GCCTAGAGGG	CCAGGACAAA	GGAGACCACC	GCCTCCACCA	CCACCTCCAT	CACCACTACC	240
ACCGCCTTCC	CAACCACCCC	CGCTGCCTCC	ACTAAGCCCT	CTAAAGAAGA	AGGACAACAT	300
AGAGCTGTGG	CTACCGGTGA	TATTTTTTCAT	GGTGTCTGGT	GCTCTGGTTG	GAATGGGGTT	360
AGGAATGTAT	CAACTCTTTC	ATCTACAGAA	GGAAGTGGCA	GAAGTCCGTG	AGTTCACCAA	420
CCACAGCCTT	AGAGTATCAT	CTTTTGAAAA	GCAATAGGCC	AACCCAGCA	CACCTCTGA	480
AACCAAAAAG	CCAAGGAGTG	TGGCCCACTT	AACAGGGAAC	CCCCGCTCAA	GGTCCATCCC	540
TCTGGAATGG	GAAGACACAT	ATGGAAGTGC	TTTGATCTCT	GGAGTGAAGT	ATAAGAAAGG	600
CGGCCTTGTT	ATCAATGAGG	CTGGGTGTGA	CTTCGTATAT	TCCAAAGTAT	ACTTCCGGGG	660

2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023 2024 2025 2026 2027 2028 2029 2030 2031 2032 2033 2034 2035 2036 2037 2038 2039 2040 2041 2042 2043 2044 2045 2046 2047 2048 2049 2050 2051 2052 2053 2054 2055 2056 2057 2058 2059 2060 2061 2062 2063 2064 2065 2066 2067 2068 2069 2070 2071 2072 2073 2074 2075 2076 2077 2078 2079 2080 2081 2082 2083 2084 2085 2086 2087 2088 2089 2090 2091 2092 2093 2094 2095 2096 2097 2098 2099 2100 2101 2102 2103 2104 2105 2106 2107 2108 2109 2110 2111 2112 2113 2114 2115 2116 2117 2118 2119 2120 2121 2122 2123 2124 2125 2126 2127 2128 2129 2130 2131 2132 2133 2134 2135 2136 2137 2138 2139 2140 2141 2142 2143 2144 2145 2146 2147 2148 2149 2150 2151 2152 2153 2154 2155 2156 2157 2158 2159 2160 2161 2162 2163 2164 2165 2166 2167 2168 2169 2170 2171 2172 2173 2174 2175 2176 2177 2178 2179 2180 2181 2182 2183 2184 2185 2186 2187 2188 2189 2190 2191 2192 2193 2194 2195 2196 2197 2198 2199 2200 2201 2202 2203 2204 2205 2206 2207 2208 2209 2210 2211 2212 2213 2214 2215 2216 2217 2218 2219 2220 2221 2222 2223 2224 2225 2226 2227 2228 2229 2230 2231 2232 2233 2234 2235 2236 2237 2238 2239 2240 2241 2242 2243 2244 2245 2246 2247 2248 2249 2250 2251 2252 2253 2254 2255 2256 2257 2258 2259 2260 2261 2262 2263 2264 2265 2266 2267 2268 2269 2270 2271 2272 2273 2274 2275 2276 2277 2278 2279 2280 2281 2282 2283 2284 2285 2286 2287 2288 2289 2290 2291 2292 2293 2294 2295 2296 2297 2298 2299 2300 2301 2302 2303 2304 2305 2306 2307 2308 2309 2310 2311 2312 2313 2314 2315 2316 2317 2318 2319 2320 2321 2322 2323 2324 2325 2326 2327 2328 2329 2330 2331 2332 2333 2334 2335 2336 2337 2338 2339 2340 2341 2342 2343 2344 2345 2346 2347 2348 2349 2350 2351 2352 2353 2354 2355 2356 2357 2358 2359 2360 2361 2362 2363 2364 2365 2366 2367 2368 2369 2370 2371 2372 2373 2374 2375 2376 2377 2378 2379 2380 2381 2382 2383 2384 2385 2386 2387 2388 2389 2390 2391 2392 2393 2394 2395 2396 2397 2398 2399 2400 2401 2402 2403 2404 2405 2406 2407 2408 2409 2410 2411 2412 2413 2414 2415 2416 2417 2418 2419 2420 2421 2422 2423 2424 2425 2426 2427 2428 2429 2430 2431 2432 2433 2434 2435 2436 2437 2438 2439 2440 2441 2442 2443 2444 2445 2446 2447 2448 2449 2450 2451 2452 2453 2454 2455 2456 2457 2458 2459 2460 2461 2462 2463 2464 2465 2466 2467 2468 2469 2470 2471 2472 2473 2474 2475 2476 2477 2478 2479 2480 2481 2482 2483 2484 2485 2486 2487 2488 2489 2490 2491 2492 2493 2494 2495 2496 2497 2498 2499 2500 2501 2502 2503 2504 2505 2506 2507 2508 2509 2510 2511 2512 2513 2514 2515 2516 2517 2518 2519 2520 2521 2522 2523 2524 2525 2526 2527 2528 2529 2530 2531 2532 2533 2534 2535 2536 2537 2538 2539 2540 2541 2542 2543 2544 2545 2546 2547 2548 2549 2550 2551 2552 2553 2554 2555 2556 2557 2558 2559 2560 2561 2562 2563 2564 2565 2566 2567 2568 2569 2570 2571 2572 2573 2574 2575 2576 2577 2578 2579 2580 2581 2582 2583 2584 2585 2586 2587 2588 2589 2590 2591 2592 2593 2594 2595 2596 2597 2598 2599 2600 2601 2602 2603 2604 2605 2606 2607 2608 2609 2610 2611 2612 2613 2614 2615 2616 2617 2618 2619 2620 2621 2622 2623 2624 2625 2626 2627 2628 2629 2630 2631 2632 2633 2634 2635 2636 2637 2638 2639 2640 2641 2642 2643 2644 2645 2646 2647 2648 2649 2650 2651 2652 2653 2654 2655 2656 2657 2658 2659 2660 2661 2662 2663 2664 2665 2666 2667 2668 2669 2670 2671 2672 2673 2674 2675 2676 2677 2678 2679 2680 2681 2682 2683 2684 2685 2686 2687 2688 2689 2690 2691 2692 2693 2694 2695 2696 2697 2698 2699 2700 2701 2702 2703 2704 2705 2706 2707 2708 2709 2710 2711 2712 2713 2714 2715 2716 2717 2718 2719 2720 2721 2722 2723 2724 2725 2726 2727 2728 2729 2730 2731 2732 2733 2734 2735 2736 2737 2738 2739 2740 2741 2742 2743 2744 2745 2746 2747 2748 2749 2750 2751 2752 2753 2754 2755 2756 2757 2758 2759 2760 2761 2762 2763 2764 2765 2766 2767 2768 2769 2770 2771 2772 2773 2774 2775 2776 2777 2778 2779 2780 2781 2782 2783 2784 2785 2786 2787 2788 2789 2790 2791 2792 2793 2794 2795 2796 2797 2798 2799 2800 2801 2802 2803 2804 2805 2806 2807 2808 2809 2810 2811 2812 2813 2814 2815 2816 2817 2

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GTTAAGCTTT TCAGTCAGCA TGATAGAA 28

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GTTTCTAGAT CAGAGTTTGA GTAAGCC 27

## (2) INFORMATION FOR SEQ ID NO: 28:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CCAAGACTAG TTAACACAGC ATGATCGAAA

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## (2) INFORMATION FOR SEQ ID NO: 29:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CCAATGCGGC CGCACTCAGA ATTCAACCTG

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## (2) INFORMATION FOR SEQ ID NO: 30:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 972 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

TCTAGACTCA	GGA	CTGAGAA	GAAGTAA	AAC	CGTTTGCTGG	GGCTGGCCTG	ACTCACCAGC	60
TGCCATGCAG	CAGCCCTTCA	ATTACCCATA	TCCCCAGATC	TACTGGGTGG	ACAGCAGTGC			120
CAGCTCTCCC	TGGGCCCTC	CAGGCACAGT	TCTTCCCTGT	CCAACCTCTG	TGCCCAGAAG			180
GCCTGGTCAA	AGGAGGCCAC	CACCACCACC	GCCACCGCCA	CCACTACCAC	CTCCGCCGCC			240
GCCGCCACCA	CTGCCTCCAC	TACCGCTGCC	ACCCCTGAAG	AAGAGAGGGA	ACCACAGCAC			300
AGGCCTGTGT	CTCCTTGTGA	TGTTTTTCAT	GGTTCCTGGTT	GCCTTGGTAG	GATTGGGCCT			360
GGGGATGTTT	CAGCTCTTCC	ACCTACAGAA	GGAGCTGGCA	GAACTCCGAG	AGTCTACCAG			420
CCAGATGCAC	ACAGCATCAT	CTTTGGAGAA	GCAAATAGGC	CACCCAGTC	CACCCCTGA			480

AAAAAAGGAG	CTGAGGAAAG	TGGCCCATT	AACAGGCAAG	TCCAAC TCA	GGTCCATGCC	540
TCTGGAATGG	GAAGACACCT	ATGGAATTGT	CCTGCTTTCT	GGAGTGAAGT	ATAAGAAGGG	600
TGGCCTTG TG	ATCAATGAAA	CTGGGCTGTA	CTTTGTATAT	TCCAAAGTAT	ACTTCCGGGG	660
TCAATCTTGC	AACAACCTGC	CCCTGAGCCA	CAAGGTCTAC	ATGAGGAAC T	CTAAGTATCC	720
CCAGGATCTG	GTGATGATGG	AGGGGAAGAT	GATGAGCTAC	TGCACTACTG	GGCAGATGTG	780
GGCCCGCAGC	AGCTACCTGG	GGGCAGTGTT	CAATCTTACC	AGTGCTGATC	ATTTATATGT	840
CAACGTATCT	GAGCTCTCTC	TGGTCAATTT	TGAGGAATCT	CAGACGTTTT	TCGGCTTATA	900
TAAGCTCTAA	GAGAAGCACT	TTGGGATTCT	TTCCATTATG	ATTCTTTGTT	ACAGGCACCG	960
AGATGTTCTA	GA					972

## (2) INFORMATION FOR SEQ ID NO: 31:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	885 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ATGCAGCAGC	CCATGAATTA	CCCATGTCCC	CAGATCTTCT	GGGTAGACAG	CAGTGCCACT	60
TCATCTTGGG	CTCCTCCAGG	GTCAGTTTTT	CCCTGTCCAT	CTTGTGGGCC	TAGAGGGCCG	120
GACCAAAGGA	GACCGCCACC	TCCACCACCA	CCTGTGTCAC	CACTACCACC	GCCATCACAA	180
CCACTCCCAC	TGCCGCCACT	GACCCCTCTA	AAGAAGAAGG	ACCACAACAC	AAATCTGTGG	240
CTACCGGTGG	TATTTTTTCAT	GGTTCTGGTG	GCTCTGGTTG	GAATGGGATT	AGGAATGTAT	300
CAGCTCTTCC	ACCTGCAGAA	GGAAGTGGCA	GAAGTCCGTG	AGTTCACCAA	CCAAAGCCTT	360
AAAGTATCAT	CTTTTGAAAA	GCAAATAGCC	AACCCACGTA	CACCCTCTGA	AAAAAAAGAG	420
CCGAGGAGTG	TGGCCCATT	AACAGGGAAC	CCCCACTCAA	GGTCCATCCC	TCTGGAATGG	480
GAAGACACAT	ATGGAACCGC	TCTGATCTCT	GGAGTGAAGT	ATAAGAAAGG	TGGCCTTG TG	540
ATCAACGAAG	CTGGGTTGTA	CTTCGTATAT	TCCAAAGTAT	ACTTCCGGGG	TCAGTCTTGC	600
AACAACCCAGC	CCCTAAACCA	CAAGGTCTAT	ATGAGGAAC T	CTAAGTATCC	TGGGGATCTG	660
GTGCTAATGG	AGGAGAAGAG	GTTGAACTAC	TGCACTACTG	GACAGATATG	GGCCACAGC	720
AGCTACCTGG	GGGCAGTATT	CAATCTTACC	AGTGCTGACC	ATTTATATGT	CAACATATCT	780
CAACTCTCTC	TGATCAATTT	TGAGGAATCT	AAGACCTTTT	TCGGCTTGTA	TAAGCTTTAA	840
AAGAAAAAGC	ATTTTAAAAT	GATCTACTAT	TCTTTATCAT	GGGCA		885

## (2) INFORMATION FOR SEQ ID NO: 32:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	29 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear



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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 680 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

ATGCCGGAGG	AAGGTCGCCC	TTGCCCTTGG	GTTCGCTGGA	GCGGGACCGC	GTTCCAGCGC	60
CAATGGCCAT	GGCTGCTGCT	GGTGGTGT	ATTACTGTGT	TTTGCTGT	GTTTCATTGT	120
AGCGGACTAC	TCAGTAAGCA	GCAACAGAGG	CTGCTGGAGC	ACCTTGAGCC	GCACACAGCT	180
GAGTTACAGC	TGAATCTCAC	AGTTCCCTCGG	AAGGACCCCA	CAC TGCGCTG	GGGAGCAGGC	240
CCAGCCTTGG	GAAGGTCCTT	CACACACGGA	CCAGAGCTGG	AGGAGGGCCA	TCTGCGTATC	300
CATCAAGATG	GCCCTCTACAG	GCTGCATATC	CAGGTGACAC	TGGCCAAC TG	CTCTTCCCCA	360
GGCAGCACCC	TGCAGCACAG	GGCCACCC TG	GCTGTGGGCA	TCTGCTCCCC	CGCTGCGCAC	420
GGCATCAGCT	TGCTGCGTGG	GCGCTTTTGG	CAGGACTGTA	CAGTGGCAT T	ACAGCGCCTG	480
ACATACTTGG	TCCACGGAGA	TGTCCTCTGT	ACCAACCTCA	CCCTGCCCTCT	GCTGCCGTCC	540
CGCAACGCTG	ATGAGACTTT	CTTTGGAGTT	CAGTGGATAT	GCCCTTGACC	ACAAC TCCAG	600
GATGACTTGT	GAATATTTTT	TTTCTTTTCA	AGTTC TACGT	ATTTATAAAT	GTATATAGTA	660
CACATAAAAA	AAAAAAAAAA					680

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 846 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEO ID NO: 37:

ATGCAGCAGC	CCTTCAATTA	CCCATATCCC	CAGATCTACT	GGGTGGACAG	CAGTGCCAGC	60
TCTCCCTGGG	CCCCTCCAGG	CACAGTTCTT	CCCTGTCCAA	CTCTGTGTC	CAGAAGGCCT	120
GGTCAAAGGA	GGCCACCACC	ACCACCGCCA	CCGCCACCAC	TACCACCTCC	GCCGCCGCCG	180
CCACCAC'TGC	CTCCAC'TACC	GCTGCCACCC	CTGAAGAAGA	GAGGGAACCA	CAGCACAGGC	240
CTGTGTCTCC	TTGTGATGTT	TTTCATGGTT	CTGGTTGCC'T	TGGTAGGATT	GGGCCTGGGG	300
ATGTTTCAGC	TCTTCCACCT	GCAGAAGGAA	CTGGCAGAAC	TCCGTGAGTT	CACCAACCAA	360
AGCCTTAAAG	TATCATCTTT	TGAAAAGCAA	ATAGGCCACC	CCAGTCCACC	CCCTGAAAAA	420
AAGGAGCTGA	GGAAAGTGGC	CCATTTTAACA	GGCAAGTCCA	ACTCAAGGTC	CATGCC'TCTG	480
GAATGGGAAG	ACACCTATGG	AATTGTCC'TG	CTTTCTGGAG	TGAAGTATAA	GAAGGGTGGC	540
CTTGTGATCA	ATGAAACTGG	GCTGTACTTT	GTATATTCCA	AAGTATACTT	CCGGGGTCAA	600
TCTTGCAACA	ACCTGCCCC'T	GAGCCACAAG	GTCTACATGA	GGAACTCTAA	GTATCCCCAG	660

**Table 1**

Parameter	Value
Number of subjects	20
Age (years)	22-35
Gender	Males
Height (cm)	170-185
Weight (kg)	65-85
BMI (kg/m <sup>2</sup> )	22-25
VO <sub>2</sub> max (ml/min/kg)	45-55
Resting heart rate (b/min)	60-70
Maximal heart rate (b/min)	180-190
Exercise intensity (% VO <sub>2</sub> max)	40-60
Duration of exercise (min)	30-60
Recovery time (min)	10-20
Measurement error (%)	<5

080827 090107

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 786 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

ATGCAGCAGC	CCTTCAATTA	CCCATATCCC	CAGATCTACT	GGGTGGACAG	CAGTGCCAGC	60
TCTCCCTGGG	CCCCCTCCAGG	CACAGTTCTT	CCCTGTCCAA	CCTCTGTGCC	CAGAAGGCCT	120
GGTCAAAGGA	GGCCACCACC	ACCACCGCCA	CCGCCACCAC	TACCACCTCC	GCCGCCGCCG	180
CCACCACCTGC	CTCCACTACC	GCTGCCACCC	CTGAAGAAGA	GAGGGAACCA	CAGCACAGGC	240
CTGTGTCTCC	TTGTGATGTT	TTTCATGGTT	CTGGTTGCCCT	TGGTAGGATT	GGGCCCTGGGG	300
ATGTTTCAGC	TCTTCCGCTT	CGCACAGGCT	ATAGGCCACC	CCAGTCCACC	CCCTGAAAAA	360
AAGGAGCTGA	GGAAAGTGGC	CCATTTAACA	GGCAAGTCCA	ACTCAAGGTC	CATGCCCTCTG	420
GAATGGGAAG	ACACCTATGG	AATTTGTCCTG	CTTTCCTGGAG	TGAAGTATAA	GAAGGGTGGC	480
CTTGTGATCA	ATGAAACTGG	GCTGTACTTT	GTATATTCCA	AAGTATACTT	CCGGGGTCAA	540
TCTTGCAACA	ACCTGCCCTT	GAGCCACAAG	GTCACATGA	GGAACCTCTAA	GTATCCCCAG	600
GATCTGGTGA	TGATGGAGGG	GAAGATGATG	AGCTACTGCA	CTACTGGGCA	GATGTGGGCC	660
CGCAGCAGCT	ACCTGGGGGC	AGTGTCTCAAT	CTTACCACTG	CTGATCATTT	ATATGTCAAC	720
GTATCTGAGC	TCTCTCTGGT	CAATTTTGAG	GAATCTCAGA	CGTTTTTTCGG	CTTATATAAG	780
CTCTAA						786

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 864 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

ATGCAGCAGC	CCTTCAATTA	CCCATATCCC	CAGATCTACT	GGGTGGACAG	CAGTGCCAGC	60
TCTCCCTGGG	CCCCTCCAGG	CACAGTTCTT	CCCTGTCCAA	CCTCTGTGCC	CAGAAGGCCT	120
GGTCAAAGGA	GGCCACCACC	ACCACCGCCA	CCGCCACCAC	TACCACCTCC	GCCGCCGCCG	180
CCACCACTGC	CTCCACTACC	GCTGCCACCC	CTGAAGAAGA	GAGGGAACCA	CAGCACAGGC	240

CTGTGTCTCC	TTGTGATGTT	TTTCATGGTT	CTGGTTGCCT	TGGTAGGATT	GGGCCTGGGG	300
ATGTTTCAGC	TCTTCCAATC	CTCCATCCTC	CCCTATGCCG	GAGGAGGGTT	CGGGCTGCTC	360
GGTGCGGCGC	AGGCCCTATG	GGTGCGTCCT	GCGGCCATCC	TCAATCCTAT	AGGCCACCCC	420
AGTCCACCCC	CTGAAAAAAA	GGAGCTGAGG	AAAGTGGCCC	ATTTAACAGG	CAAGTCCAAC	480
TCAAGGTCCA	TGCCTCTGGA	ATGGGAAGAC	ACCTATGGAA	TTGTCTTGCT	TTCTGGAGTG	540
AAGTATAAGA	AGGGTGGCCT	TGTGATCAAT	GAAACTGGGC	TGTACTTTGT	ATATTCCAAA	600
GTATACTTCC	GGGGTCAATC	TTGCAACAAC	CTGCCCCCTGA	GCCACAAGGT	CTACATGAGG	660
AACTCTAAGT	ATCCCCAGGA	TCTGGTGATG	ATGGAGGGGA	AGATGATGAG	CTACTGCACT	720
ACTGGGCAGA	TGTGGGCCCC	CAGCAGCTAC	CTGGGGGCAG	TGTTCAATCT	TACCAGTGCT	780
GATCATTTAT	ATGTCAACGT	ATCTGAGCTC	TCTCTGGTCA	ATTTTGAGGA	ATCTCAGACG	840
TTTTTCGGCT	TATATAAGCT	CTAA				864

## (2) INFORMATION FOR SEQ ID NO: 40:

## (i) SEQUENCE CHARACTERISTICS:

(A)	LENGTH:	828 base pairs
(B)	TYPE:	nucleic acid
(C)	STRANDEDNESS:	single
(D)	TOPOLOGY:	linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

ATGCAGCAGC	CCTTCAATTA	CCCATATCCC	CAGATCTACT	GGGTGGACAG	CAGTGCCAGC	60
TCTCCCTGGG	CCCCTCCAGG	CACAGTTCTT	CCCTGTCCAA	CCTCTGTGCC	CAGAAGGCCT	120
GGTCAAAGGA	GGCCACCACC	ACCACCGCCA	CCGCCACCAC	TACCACCTCC	GCCGCCGCCG	180
CCACCACTGC	CTCCACTACC	GCTGCCACCC	CTGAAGAAGA	GAGGGAACCA	CAGCACAGGC	240
CTGTGTCTCC	TTGTGATGTT	TTTCATGGTT	CTGGTTGCCT	TGGTAGGATT	GGGCCTGGGG	300
ATGTTTCAGC	TCTTCCACCT	ACAGCGAGAG	TCTACCAGCC	AGATGCACAC	AGCATCATCT	360
TTGGAGAAGC	AAATAGGCCA	CCCCAGTCCA	CCCCCTGAAA	AAAAGGAGCT	GAGGAAAGTG	420
GCCCATTTAA	CAGGCAAGTC	CAACTCAAGG	TCCATGCCTC	TGGAATGGGA	AGACACCTAT	480
GGAATTGTCC	TGCTTTCTGG	AGTGAAGTAT	AAGAAGGGTG	GCCTTGTGAT	CAATGAAACT	540
GGGCTGTACT	TTGTATATTC	CAAAGTATAC	TTCCGGGGTC	AATCTTGCAA	CAACCTGCCC	600
CTGAGCCACA	AGGTCTACAT	GAGGAAGTCT	AAGTATCCCC	AGGATCTGGT	GATGATGGAG	660
GGGAAGATGA	TGAGCTACTG	CACTACTGGG	CAGATGTGGG	CCCGCAGCAG	CTACCTGGGG	720
GCAAGTGTCA	ATCTTACCAG	TGCTGATCAT	TTATATGTCA	ACGTATCTGA	GCTCTCTCTG	780
GTCAATTTTG	AGGAATCTCA	GACGTTTTTC	GGCTTATATA	AGCTCTAA		828

## (2) INFORMATION FOR SEQ ID NO: 41:

## (i) SEQUENCE CHARACTERISTICS:

(A)	LENGTH:	846 base pairs
(B)	TYPE:	nucleic acid
(C)	STRANDEDNESS:	single
(D)	TOPOLOGY:	linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

ATGGCTATGA	TGGAGGTCCA	GGGGGGACCC	AGCCTGGGAC	AGACCTGCGT	GCTGATCGTG	60
ATCTTCACAG	TGCTCCTGCA	GTCTCTCTGT	GTGGCTGTAA	CTTACGTGTA	CTTTACCAAC	120
GAGCTGAAGC	AGATGCAGGA	CAAGTACTCC	AAAAGTGGCA	TTGCTTGTTT	CTTAAAAGAA	180
GATGACAGTT	ATTGGGACCC	CAATGACGAA	GAGAGTATGA	ACAGCCCCTG	CTGGCAAGTC	240
AAGTGGCAAC	TCCGTCAGCT	CGTTAGAAAAG	ATGATTTTGA	GAACCTCTGA	GGAAACCATT	300
TCTACAGTTC	AAGAAAAGCA	ACAAAATATT	TCTCCCCTAG	TGAGAGAAAG	AGGTCCTCAG	360
AGAGTAGCAG	CTCACATAAC	TGGGACCAGA	GGAAGAAGCA	ACACATTGTC	TTCTCCAAAC	420
TCCAAGAATG	AAAAGGCTCT	GGGCCGCAAA	ATAAACTCCT	GGGAATCATC	AAGGAGTGGG	480
CATTCAATTCC	TGAGCAACTT	GCACTTGAGG	AATGGTGAAC	TGGTCATCCA	TGAAAAAGGG	540
TTTTACTACA	TCTATTCCCA	AACATACTTT	CGATTTTCAGG	AGGAAATAAA	AGAAAACACA	600
AAGAACGACA	AACAAATGGT	CCAATATATT	TACAAATACA	CAAGTTATCC	TGACCCTATA	660
TTGTTGATGA	AAAGTGCTAG	AAATAGTTGT	TGGTCTAAAG	ATGCAGAATA	TGGACTCTAT	720
TCCATCTATC	AAGGGGGAAT	ATTTGAGCTT	AAGGAAAATG	ACAGAAATTT	TGTTTCTGTA	780
ACAAATGAGC	ACTTGATAGA	CATGGACCAT	GAAGCCAGTT	TTTTCGGGGC	CTTTTTAGTT	840
GGCTAA						846

## (2) INFORMATION FOR SEQ ID NO: 42:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	876 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

ATGCCTTCCT	CAGGGGCCCT	GAAGGACCTC	AGCTTCAGTC	AGCACTTCAG	GATGATGGTG	60
ATTTGCATAG	TGCTCCTGCA	GGTGCTCCTG	CAGGCTGTGT	CTGTGGCTGT	GACTTACATG	120
TACTTCACCA	ACGAGATGAA	GCAGCTGCAG	GACAATTACT	CCAAAATTGG	ACTAGCTTGC	180
TTCTCAAAGA	CGGATGAGGA	TTTCTGGGAC	TCCACTGATG	GAGAGATCTT	GAACAGACCC	240
TGCTTGCAGG	TTAAGAGGCA	ACTGTATCAG	CTCATTGAAG	AGGTGACTTT	GAGAACCTTT	300
CAGGACACCA	TTTCTACAGT	TCCAGAAAAG	CAGCTAAGTA	CTCCTCCCTT	GCCCAGAGGT	360
GGAAGACCTC	AGAAAGTGGC	AGCTCACATT	ACTGGGATCA	CTCGGAGAAG	CAACTCAGCT	420
TTAATTCCAA	TCTCCAAGGA	TGGAAAGACC	TTAGGCCAGA	AGATTGAATC	CTGGGAGTCC	480
TCTCGGAAAG	GGCATTCAAT	TCTCAACCAC	GTGCTCTTTA	GGAATGGAGA	GCTGGTCATC	540
GAGCAGGAGG	GCCTGTATTA	CATCTATTCC	CAAACATACT	TCCGATTTC	GGAAGCTGAA	600
GACGCTTCCA	AGATGGTCTC	AAAGGACAAG	GTGAGAACCA	AACAGCTGGT	GCAGTACATC	660
TACAAGTACA	CCAGCTATCC	GGATCCCATA	GTGCTCATGA	AGAGCGCCAG	AAACAGCTGT	720
TGGTCCAGAG	ATGCCGAGTA	CGGACTGTAC	TCCATCTATC	AGGGAGGATT	GTTCGAGCTA	780
AAAAAAAATG	ACAGGATTTT	TGTTTCTGTG	ACAAATGAAC	ATTTGATGGA	CCTGGATCAA	840
GAAGCCAGCT	TCTTTGGAGC	CTTTTAAATT	AACTAA			876

## (2) INFORMATION FOR SEQ ID NO: 43:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 720 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

ATGGAGCCAG	GGCTGCAACA	AGCAGGCAGC	TGTGGGGGCTC	CTTCCCCTGA	CCCAGCCATG	60
CAGGTGCAGC	CCGGCTCGGT	AGCCAGCCCC	TGGAGAAGCA	CGAGGCCCTG	GAGAAGCACA	120
AGTCGCAGCT	ACTTCTACCT	CAGCACCACC	GCACTGGTGT	GCCTTGTTGT	GGCAGTGGCG	180
ATCATTTCTGG	TACTGGTAGT	CCAGAAAAAG	GACTCCACTC	CAAATACAAC	TGAGAAGGCC	240
CCCCTTAAAG	GAGGAAATTG	CTCAGAGGAT	CTCTTCTGTA	CCCTGAAAAG	TACTCCATCC	300
AAGAAGTCAT	GGGCTTACCT	CCAAGTGTC	AAGCATCTCA	ACAATACCAA	ACTGTCATGG	360
AACGAAGATG	GCACCATCCA	CGGACTCATA	TACCAGGACG	GGAACCTGAT	AGTCCAATTC	420
CCTGGCTTGT	ACTTCATCGT	TTGCCAACTG	CAGTTCCTCG	TGCAGTGCTC	AAATCATTCT	480
GTGGACCTGA	CATTGCAGCT	CCTCATCAAT	TCCAAGATCA	AAAAGCAGAC	GTTGGTAACA	540
GTGTGTGAGT	CTGGAGTTCA	GAGTAAGAAC	ATCTACCAGA	ATCTCTCTCA	GTTTTTGCTG	600
CATTACTTAC	AGGTCAACTC	TACCATATCA	GTCAGGGTGG	ATAATTTCCA	GTATGTGGAT	660
ACAAACACTT	TCCCTCTTGA	TAATGTGCTA	TCCGTCTTCT	TATATAGTAG	CTCAGACTGA	720

## (2) INFORMATION FOR SEQ ID NO: 44:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 930 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

ATGGACCAGC	ACACACTTGA	TGTGGAGGAT	ACCGCGGATG	CCAGACATCC	AGCAGGTACT	60
TCGTGCCCCCT	CGGATGCGGC	GTCCTCAGA	GATACCGGGC	TCCTCGCGGA	CGCTGCGCTC	120
CTCTCAGATA	CTGTGCGCCC	CACAAATGCC	GCGCTCCCCA	CGGATGCTGC	CTACCCTGCG	180
GTTAATGTTC	GGGATCGCGA	GGCCGCGTGG	CCGCCTGCAC	TGAACTTCTG	TTCCCGCCAC	240
CCAAAGCTCT	ATGGCCTAGT	CGCTTTGGTT	TTGCTGCTTC	TGATCGCCGC	CTGTGTTCTT	300
ATCTTCACCC	GCACCGAGCC	TCGGCCAGCG	CTCACAATCA	CCACCTCGCC	CAACCTGGGT	360
ACCCGAGAGA	ATAATGCAGA	CCAGGTCACC	CCTGTTTCCC	ACATTGGCTG	CCCCAACACT	420
ACACAACAGG	GCTCTCCTGT	GTTGCGCAAG	CTACTGGCTA	AAAACCAAGC	ATCGTTGTGC	480
AATACAACCTC	TGAAGTGGCA	CAGCCAAGAT	GGAGCTGGGA	GCTCATACCT	ATCTCAAGGT	540
CTGAGGTACG	AAGAAGACAA	AAAGGAGTTG	GTGGTAGACA	GTCCCGGGCT	CTACTACGTA	600
TTTTTTGGAAC	TGAAGCTCAG	TCCAACATTC	ACAAACACAG	GCCACAAGGT	GCAGGGCTGG	660

GTCTCTCTTG	TTTTGCAAGC	AAAGCCTCAG	GTAGATGACT	TTGACAACTT	GGCCCTGACA	720
GTGGAAGTGT	TCCCTTGCTC	CATGGAGAAC	AAGTTAGTGG	ACCGTTCCTG	GAGTCAACTG	780
TTGCTCCTGA	AGGCTGGCCA	CCGCCTCAGT	GTGGGTCTGA	GGGCTTATCT	GCATGGAGCC	840
CAGGATGCAT	ACAGAGACTG	GGAGCTGTCT	TATCCAACA	CCACCAGCTT	TGGACTCTTT	900
CTTGTGAAAC	CCGACAACCC	ATGGGAATGA				930

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